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## FERTILIZATION IN *ULMUS* WITH SPECIAL REFERENCE TO HYBRIDIZATION PROCEDURE<sup>1</sup>

BY L. P. V. JOHNSON<sup>2</sup>

### Abstract

Germination tests on fruits derived from open, self, and cross pollinations made in *Ulmus* during four seasons strongly indicate a high degree of self sterility, possibly due to protogyny. It is suggested that a hybridization procedure involving the cross pollination of non-emasculated flowers is feasible in many species of the genus, especially under conditions that permit large-scale crossing and the growing of fairly extensive populations from which selfed seedlings may be eliminated.

### Introduction

The flowers of *Ulmus*, the elm, are small, perfect, and occur in close clusters. These characteristics proved a serious handicap in hybridization work based on the emasculation procedure. Since *Ulmus* is reported to be normally cross fertilized and to exhibit marked protogyny (1, 2) there appeared to be some prospects of finding a degree of self sterility\* sufficient to obviate the necessity of emasculation. This led to the experiments reported herein.

### Materials and Methods

The study was made upon a number of elms growing in roughly the same site at the Dominion Arboretum, Ottawa. The species involved were as follows: *Ulmus americana* L., *U. fulva* Michx.,  $\times$  *U. hollandica* Mill. (one specimen apparently var. *major* (Sm.) Rehd., another apparently var. *superba* (Morr.) Rehd.), *U. laevis* Pall., *U. procera* Salisb., and *U. pumila* L.

Three individuals of *U. americana* and two of *U. pumila* were used as maternal parents. Only single specimens of the other species and varieties occur in the Arboretum.

Several different kinds of covering were used in bagging, the most common being a glassine paper bag protected by a kraft paper bag. Cross pollination was effected by placing a few dehiscing flower clusters from a tree of the appropriate species within the bag. Seeds were germinated on moist blotters at 22° C.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry, N.R.C. No. 1374.

<sup>2</sup> Geneticist.

\* "Self sterility" is used in the present paper to denote the practical condition of failure to self fertilize, whether due to self incompatibility or to protogyny (female part of flower maturing before male part).

## Results

The experimental results are presented in Table I. The relative percentages of viable seeds obtained from the different types of fertilization strongly indicate a high degree of self sterility in each of the *Ulmus* species studied.

A measure of the effect of bagging on seed set and seed viability is provided by the data from cross pollinations. It is believed that the flowers bagged for selfing developed under conditions comparable in every way to those provided for flowers bagged for crossing—except that self pollen only was available.

It is important to note that the viability of seeds derived from open pollinations varies greatly from year to year. This is believed to be related to seasonal differences in the conditions that promote cross fertilization (rain-free periods, winds, etc.). An observation supporting this view was made during the flowering period in 1945—a period of almost continuous rain and high humidity. Two trees,  $\times$  *U. hollandica* var. *superba* and *U. pumila*, grow side by side in the Arboretum. The side of  $\times$  *U. hollandica* next to, and with some branches touching, *U. pumila* was heavily laden with viable seeds, while the other side produced only a light yield of poor seed. Presumably, under weather conditions that permitted little pollen dissemination, the tree, being largely self sterile, was able to set viable seed only on the side with a close-at-hand source of suitable foreign pollen.

TABLE I

GERMINATION RESULTS FOR SEEDS DERIVED FROM OPEN, SELF, AND CROSS POLLINATIONS IN *Ulmus* SPECIES

Material	Total No. of:		Viable seeds, %				
	Bags	Fruits*	1939	1940	1941	1944	Average
<i>U. americana</i> , open pollinated	—	487	30.8	36.0	46.4	4.1	27.7
selfed	53	1582	0.2	3.6	3.5	0.0	1.5
$\times$ <i>U. americana</i>	29	947	56.7	33.3	23.0	2.6	25.6
<i>U. fulva</i> , open pollinated	—	200	1.6	19.6	—	—	10.6
selfed	10	179	0.0	0.0	—	—	0.0
$\times$ <i>U. americana</i>	10	261	7.7	6.2	—	—	7.1
<i>U. laevis</i> , open pollinated	—	348	24.0	70.0	35.4	3.0	31.7
selfed	18	540	0.0	0.0	1.3	0.5	0.4
$\times$ <i>U. americana</i>	13	827	58.4	42.0	27.8	1.2	35.9
<i>U. procera</i> , open pollinated	—	70	—	—	—	6.0	6.0
selfed	8	40	—	—	—	0.0	0.0
$\times$ <i>U. pumila</i>	6	52	—	—	—	13.4	13.4
<i>U. pumila</i> , open pollinated	—	400	8.0	22.0	5.0	53.0	22.0
selfed	38	1268	0.0	0.0	0.7	2.3	0.9
$\times$ ( $\times$ ) <i>U. hollandica</i> major	33	1159	6.1	22.8	4.8	12.1	10.9

\* Includes all fruits collected whether empty or fully developed.

### Discussion and Conclusions

The objective of the work, to find if self sterility existed in *Ulmus* to a degree sufficient to obviate the necessity for emasculation in the hybridization technique, has been attained. It is concluded that such a degree of self sterility does exist. The fact that elm seedlings of undoubted hybridity have been produced by bagging and cross pollinating non-emasculated flowers speaks for itself.

Whether this self sterility represents true self incompatibility or is merely due to protogyny is left an open question.

It has been observed, however, that the proportion of selfed seedlings in populations derived from interspecific cross pollinations is higher than would be expected on the basis of the results in Table I. This is attributed to some stimulatory effect on the stigma, produced by foreign pollen not entirely compatible with the maternal species, resulting in increased receptivity to self pollen.

The practical conclusion is that a hybridization procedure involving non-emasculated flowers may be recommended for *Ulmus*, under conditions that permit large-scale crossing and the growing of fairly extensive populations from which the selfed seedlings may be eliminated.

### Acknowledgments

The author acknowledges the assistance of Mr. E. C. Bradley in the field work of the investigation, of Dr. H. A. Senn in identifying the material, and of Mr. M. W. Thistle and Dr. Margaret L. Landes in the preparation of the manuscript.

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# TETRAPLOIDY IN FLAX<sup>1</sup>

By J. G. ROSS<sup>2</sup> AND J. W. BOYES<sup>3</sup>

## Abstract

Tetraploid forms of the flax variety, Redwing, and the F<sub>1</sub> flax hybrid, Bison × Redwing, were most easily obtained by treatment of the epicotyl of seedlings with colchicine in lanolin emulsion. Seeds of the tetraploid forms were considerably larger than those of the diploid; but, because of their lower fertility and later maturity, yield and quality were definitely inferior.

## Introduction

Doubling the chromosome number of plants by means of colchicine treatment was investigated as a means of obtaining a large-seeded oil flax adapted to conditions in western Canada. Increased seed size of the tetraploid over the diploid forms has been reported in flax (3) and in numerous other plant species (1, 2). Fertility, however, may be decreased in autotetraploid forms. Though this possibility was recognized, the ease with which tetraploid plants can be obtained prompted the present investigation.

## Results

Plants of the variety Redwing and the F<sub>1</sub> hybrid of the cross Bison × Redwing were obtained in the tetraploid form after colchicine treatment of large numbers of seeds and seedlings. The application of colchicine in lanolin emulsion to the epicotyl of very young seedlings gave a larger proportion of tetraploid plants than did any other method. Increase in the size of pollen and stomata, characteristic of tetraploid plants, as shown in Table I, was used as a means of identifying the tetraploids. The identity of these plants was confirmed by chromosome counts: diploid plants had 30 chromosomes, while the tetraploid plants had approximately 60. The majority of these tetraploid plants had the exact  $4n$  number of 60 chromosomes.

Comparisons of the tetraploids with the diploids were made in 1942, 1943, and 1944 and are shown in Tables I, II, and III. In 1944, though their number of days to blooming was not consistently greater, the tetraploids matured much later than did the diploids. In that year, results also indicated that the tetraploids had a tendency toward weaker straw and were slightly shorter than the diploid forms. Flower size and seed size were considerably larger in the tetraploids, but seed set was greatly reduced. This is shown in Tables I and II by the low average number of tetraploid seeds per boll, and in Table III by the low yields of the tetraploids. In the quality tests conducted in 1943 and 1944 the tetraploids, in comparison with the diploids,

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were definitely inferior in oil content, equal or slightly inferior in drying quality, and approximately equal in percentage nitrogen. Though seed size was increased by doubling the chromosome number, as is shown by the larger

TABLE I  
COMPARISON OF TETRAPLOID AND DIPLOID FLAX PLANTS OF THE VARIETY  
REDWING, GROWN IN THE GREENHOUSE IN 1942

	No. of plants	No. of days to first flower	Flower diameter, mm.	Pollen diameter, $m\mu$	Stomatal length, $m\mu$	Height at maturity, in.	No. of seeds per boll	1000-kernel weight, gm.
Diploids	14	163	20	69	33	26.4	2.0	5.64
Tetraploids	13	144	14	55	25	27.4	7.1	4.78

TABLE II  
COMPARISON OF SEED SET OF DIPLOID AND TETRAPLOID SHOOTS OF THE SAME PLANTS  
GROWN IN THE GREENHOUSE IN 1942 AND IN THE FIELD IN 1943

Variety	Diploid		Tetraploid	
	No. of bolls	Average no. of seeds per boll	No. of bolls	Average no. of seeds
1942				
Redwing	6		4	2.1
Bison $\times$ Redwing	4		21	3.0
1943				
Redwing	200	8.0	200	3.0
Bison $\times$ Redwing	—	—	200	2.8

TABLE III  
COMPARISON OF PLANT AND SEED CHARACTERISTICS OF TETRAPLOID AND  
DIPLOID FORMS OF FLAX IN THE YEARS 1943 AND 1944

Variety	Days to full bloom	Straw strength, (1-10)	Height, in.	Days to maturity	Yield, bu. per acre	Oil content, % dry basis	Iodine value, Wij's	1000-kernel weight, gm.	Nitrogen, % dry basis
1943									
Redwing 4n	—	—	—	—	—	35.7	190.5	6.77	4.29
Redwing 2n	—	—	—	—	—	39.5	196.8	5.07	4.19
1944									
Redwing 4n	56	8.0	24.0	127	10.4	36.9	192.7	—	4.24
Redwing 2n	53	10.0	25.2	99	30.0	40.5	193.6	—	4.25
Redwing $\times$ Bison 4n	54	9.0	23.0	121	10.4	37.3	193.9	—	4.43
Redwing $\times$ Bison 2n	57	8.5	25.2	109	25.2	40.2	193.1	—	4.26

size of tetraploid seed (Fig. 1) and by the increased 1000-kernel weight (Tables I and III), yet late and uneven maturity of the tetraploids led to the formation of many immature green seeds. Consequently the oil content was severely reduced.

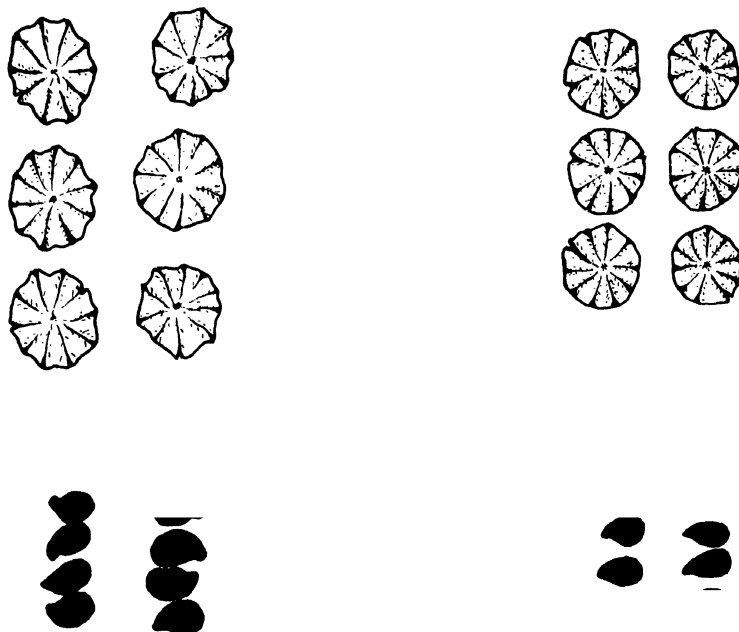


FIG. 1. Illustration of the difference in size of flax bolls and seeds from a tetraploid plant on the left and from a diploid plant on the right. ( $1\frac{1}{2} \times$  natural size).

These results show that, even though seed size of flax may be increased by doubling the chromosome number, the consequent low fertility and late maturity of tetraploids prevent the immediate development of a more suitable variety by this method. It is possible, however, that fertility may be increased by selection.

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# GERMINATION OF SEEDS OF THREE SPECIES OF *AGROSTIS*<sup>1</sup>

BY C. W. LEGGATT<sup>2</sup>

## Abstract

Germination tests on seed of the 1939 crop of *Agrostis stolonifera* L. var. *compacta* Hartm., *A. tenuis* Sibth., and *A. canina* L. were begun in September of the same year and carried through to May, 1940, with the object of studying the drift, with time, of germination capacity and germination speed under different conditions.

Seeds of the three species proved to be highly light-sensitive but low light-requiring. Germination capacity was proportional to light intensity while germination speed was inversely proportional to light intensity. The possibility that excess of light (>200 ft.-c. for seven and one-half hours daily) might depress germination capacity was suggested. There was no significant difference, in their effect on germination, between Mazda and Fluorescent "Daylite" illumination at equal foot candle intensity.

Potassium nitrate in 0.2% solution proved more effective than light in promoting germination in not fully germinating-ripe seeds but its use did not stimulate germination beyond the natural limits of fully ripe seed; thus its use in seed control laboratories is justified for these species. Its effect on the seed is clearly distinct, physiologically, from that of light.

An interesting phenomenon was observed, consisting in a falling off in germination capacity accompanied by an absolute increase in germination speed during the period February-March.

Seeds of these species fall into four classes. The interrelationships between these classes and changes within them and their connection with the February-March phenomenon are discussed in the text.

## Introduction

The seeds of *Agrostis* have for some years been generally recognized as light-sensitive. Toole and Goss (8) in 1925 reported that the use of a 0.2% solution of potassium nitrate to moisten the substratum and exposure of the test to light during part of the day hastened the germination of some samples of *Agrostis* but that a sharp alternation of temperature daily was also necessary. In 1929 Thygeson (7) found that potassium nitrate solution gave results closely comparable to results obtained on soil in uncovered Petri dishes, the seeds being barely covered with soil, and concluded that the use of nitrate was justifiable as a germination medium for seeds of *Agrostis*.

Gadd (3) in 1939 pointed out that *Agrostis* seed in most years is not germinating-ripe on which account his laboratory puts in a double test on all samples, using an 18° to 36° C. temperature alternation for one and 10° to 36° C. for the other. Samples not germinating-ripe under standard conditions germinate very satisfactorily under this latter alternation.

<sup>1</sup> Manuscript received September 14, 1945.

Contribution from the Seed Research Laboratory, Division of Plant Products, Department of Agriculture, Ottawa.

<sup>2</sup> In charge of Seed Research.

Note:—This paper was practically ready for publication in 1942, when the author received leave of absence to engage in special war duties. An abstract of the paper was presented in the A.O.S.A. News Letter for December, 1942. In order no longer to delay publication, a review of more recent literature, than was then available, has not been made.



In the Canadian laboratories similar difficulties have been found and have frequently been the cause of failure of different tests on the same material to corroborate one another. Elliott (1) reported receiving in August, 1932, a number of samples of red top (*A. stolonifera* L. var. *major* Gaud. Far.) that were not germinating-ripe. The average germination of five samples was 74%. By November of the same year the germination had risen to 90%. In 1937 he reported receiving a sample of brown top (*A. tenuis* Sibth.), which germinated 47% on the original test, a retest giving 63% while a test using 0.2% nitrate gave 90%. On the same sample he reports the Sackville laboratory as having obtained 96% using the aluminium cup method and 88% by the standard method, on top of blotters at 20° to 30° C. in light in the Jacobsen apparatus.

As a result of the higher tests that were being obtained at Sackville than in several other laboratories in the United States and Canada, Hope (4) in 1936 conducted an investigation on the effect of full daylight compared with the subdued light that was used in some laboratories. He used samples of creeping bent grass (*A. stolonifera* L. var. *compacta* Hartm.) of New Brunswick origin and of the 1935 crop, starting the tests in June, 1936. The samples should have been fully germinating-ripe at that date but it was found necessary to prolong the tests beyond the customary time. He used both the top of blotter and the aluminium cup method under both sets of light conditions, the full daylight being given by placing the germinators close to a bright window while the subdued light was the result of placing them at the back of the room. He found that in full light the average of five samples gave 84% on top of blotters and 90% by the cup method in 35 days; in subdued light the figures were 34% and 38%, respectively after 49 days.

In a comparison of the top of blotter and cup methods he reported a mean difference with 15 samples of *Agrostis* (species not mentioned) of 10% in favour of the latter (5) and made the observation that the seedlings also appeared stronger. In 1938 (6) he reported comparisons of tests run at 30° C. constant with the standard 20° to 30° C. alternation in which the high temperature tests showed a marked inhibition.

It is clear from the foregoing that the seeds of *Agrostis* species found in commerce are highly sensitive to germinating conditions and that they retain this sensitivity long after the normal after-ripening period has been completed. Some of the results suggest that differences in light intensity have a marked effect on the germination figure such that laboratories in districts that have prolonged periods of dull weather may be unable to obtain complete germination at all times of the year. Just what are the limits in light and temperature sensitiveness have not been very clearly brought out. The investigation to be reported in this paper has been carried out with the intention of gaining further information on these points.

## Materials and Methods

Through the courtesy of Mr. A. Hope of the Sackville laboratory, a sample of creeping bent grass collected in August, 1939, on the Tantramar marshes near Sackville, N.B., and one of brown top also collected in the same month at Montecello, P.E.I., were received on the ninth day of September, 1939, for the purpose of this investigation. On the 23rd of October, 1939, a sample of velvet bent grass (*A. canina* L.) mixed with brown top grown the same year on Prince Edward Island was also received from him. The investigation was put in hand forthwith upon receipt of the material.

The material was first cleaned on the analytical blower and only such 'seeds' as contained caryopses were used.

Under each set of conditions  $2 \times 100$  seeds were used.

Three media were used; sand, Kimpak, and filter paper (Whatman No. 3), the seeds being planted on top of the medium in each case. While the sand generally gave somewhat better results, there was insufficient difference to justify separate treatment of the media in the analysis of the results. Thus the results reported are the averages of  $6 \times 100$  seeds except in the case of the velvet bent of which the separation from the brown top was so time-consuming that it was only feasible to put in  $2 \times 100$ , using sand only as the medium.

All tests were carried out in  $90 \times 15$  mm. covered Petri dishes.

Moisture was controlled by eye, just sufficient being added to give the surface of the medium a slightly shiny appearance, it having previously been found that by this means a closer control is possible than by measurement, on account of variations in the medium itself and in size and contour of the dishes. In the case of the filter paper tests, 9-cm. filter papers were placed on three thicknesses of germination blotter cut octagonally so as to leave little spaces between the sides of the octagon and the circumference of the dish. These spaces served as 'wells' to hold surplus water and helped to keep the filter papers at the most favourable moisture level.

The tests were held under conditions of controlled temperature and light. In all cases the low temperature experience lasted  $16\frac{1}{2}$  hr. daily and the high temperature seven and one-half hours. Light experience, where given, was during the high temperature period. The Mazda lamps used developed so much heat that a water heat-filter 2 to 3 in. in depth had to be employed; light intensities were measured at the seed level after passage through the filter. The tests in 'subdued light' were exposed for very brief periods daily to the light in a dark part of the room (not more than 5 ft-c.) when being transferred from one germinator to another, no particular precautions having been used to exclude light. During the high temperature experience they were exposed to light too feeble to be measured by the photronic cell used for the light measurements. The daylight fluorescent lamps employed developed so little heat that no filter was necessary. Optically, the Mazda light was quite yellow in comparison with the fluorescent.

'Dark' tests were placed in motion picture film tins as soon as planted; these were then placed in dark controlled-temperature cabinets.

It should be remarked that in the dark tests, light was completely excluded only up to the seventh day, when the first count was made. The dark tests were then opened behind a shade where there was only just enough light to see to make the count.

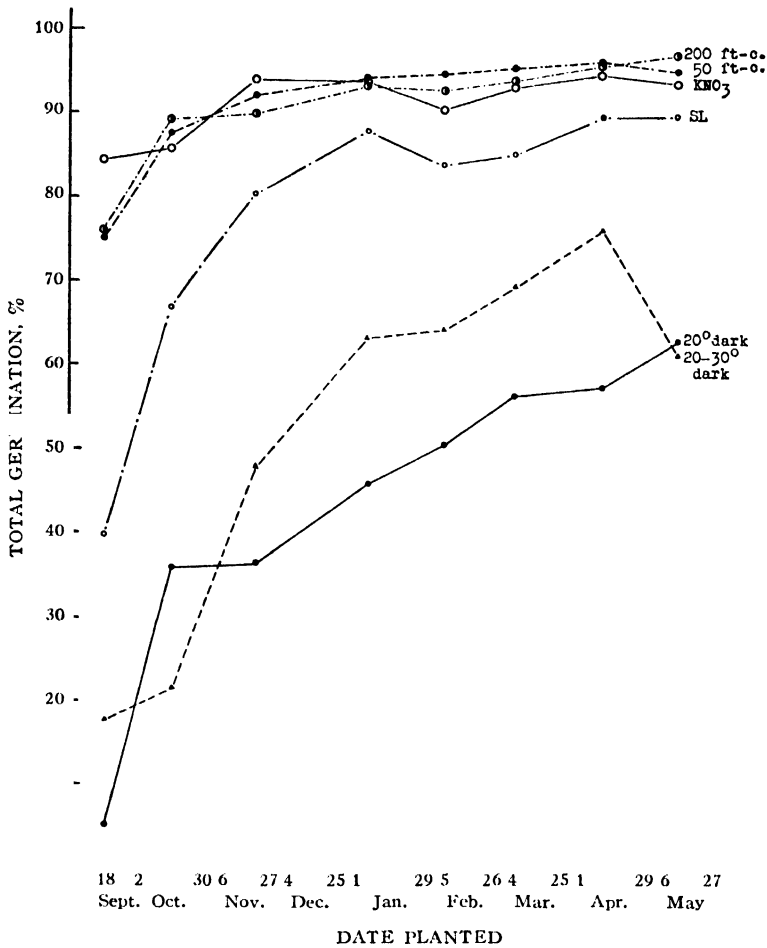


FIG. 1. *A. stolonifera* L. var. *compacta* Hartm. Total germination after 21 days of tests planted at, approximately, monthly intervals under various conditions.

Legend for Figs. 1 to 6:

- 20° C. constant temperature, dark;
- ▲—▲ 20° to 30° C., dark;
- subdued light;
- subdued light with KNO<sub>3</sub>;
- 50 ft.-c. for 7½ hr. daily;
- 200 ft.-c. for 7½ hr. daily.

All tests except the first were made at a temperature alternation of 20° C. for 16½ hr. and 30° C. for 7½ hr. daily.

Where alternating temperatures were used, the tests were transferred from a germinator held at the one temperature to one held at the other.

The following sets of conditions were used:—

- (a) 20° C. constant, dark.
- (b) 20° to 30° C., dark.
- (c) 20° to 30° C., subdued light.
- (d) 20° to 30° C., 50 ft-c., fluorescent lamps.
- (e) 20° to 30° C., 200 ft-c., Mazda lamps.
- (f) 20° to 30° C., subdued light, 0.2% nitrate solution instead of distilled water.
- (g) 20° to 30° C., 50 ft-c., Mazda lamps.

The whole series of tests was repeated at, approximately, monthly intervals. In order to distribute the work, the planting dates were staggered but the time drift was not sufficiently swift for the differences in dates to reveal appreciable differences in response. Germination counts were made at 4, 7, 10, 14, and 21 days.

## Results

### *Part 1. Drift of Germination Response with Time; Final Counts*

#### *Creeping Bent Grass*

In Fig. 1 are shown the final results, after 21 days, of samples set to germinate at various dates from 18th September, 1939, to 13th May, 1940.

The sample is of high germination capacity, a test of over 96% having been obtained at the last date of planting. It is evident, however, that it was not fully germinating-ripe by any of the methods used until the planting of 21st November when the test with nitrate in subdued light gave 94%, the highest germination obtained at that date. There is little difference between the nitrate, 50 ft-c., and 200 ft-c. tests from that date on. The tests in subdued light appear to reach their maxima by the 4th January which however is approximately 6% lower than those of the more favourable conditions, a difference that must be considered highly significant ( $P < .01$ ).

The tests in the dark reveal a marked depressive effect, which is only in small measure compensated by a temperature alternation. It should be noted, however, that the average temperature in the alternation is about 3° C. higher than the constant temperature used in the dark tests.

#### *Brown Top*

Fig. 2 gives the data for this species. This sample is also of high germination capacity, a test of nearly 95% having been obtained at the last planting. At no stage, however, was it as germinating-unripe as the creeping bent and the tests in subdued light became effectively equivalent to the tests with light or nitrate by the middle of January.

In this species also complete exclusion of light exerts a markedly depressing effect, only partially, though to a greater degree than in creeping bent, compensated by alternation of temperature.

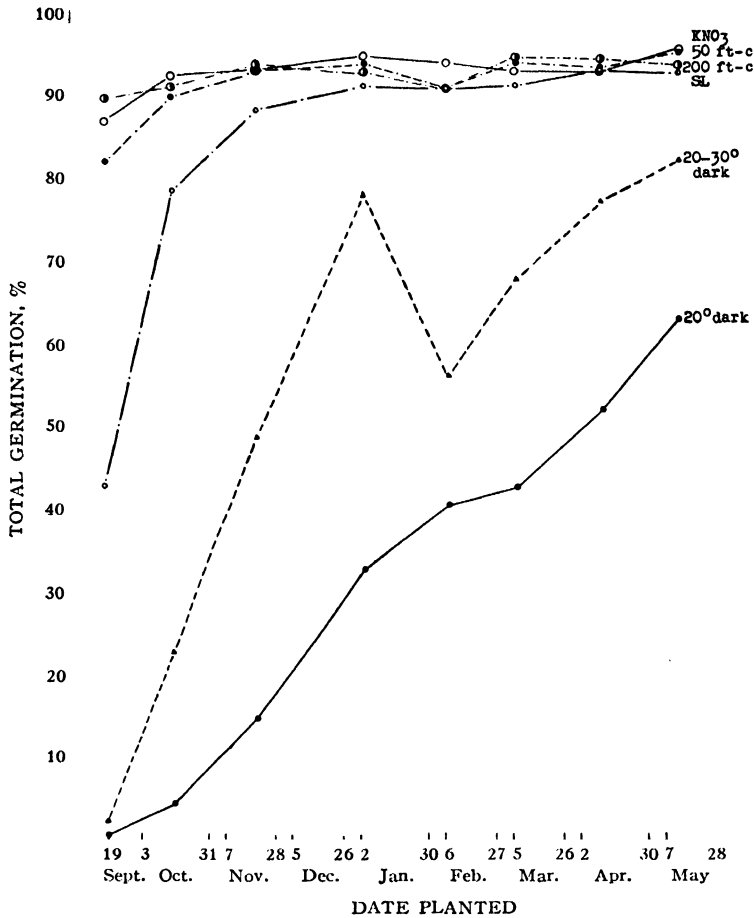


FIG. 2. *A. tenuis* Sibth. Total germination after 21 days, of tests under various conditions planted at, approximately, monthly intervals. For legend see Fig. 1.

### Velvet Bent Grass

This sample was received at a later date than the other two, so that the first date of planting is towards the end of October. Fig. 3, in which the results for this species are presented, should therefore be regarded with this in mind. It was clearly at a somewhat later stage of after-ripening at the beginning of the investigation and the results of the first series of tests are comparable with those at the same date of the creeping bent grass. The behaviour in general is intermediate between that of the creeping bent grass and the brown top. Again the depressive effect of complete exclusion of light with partial compensation brought about by alternating temperature is in evidence.

### Discussion, Part 1

A curious feature common to all these graphs, which is too consistent to be due to sampling error, is believed not to be due to the conditions of the

test since all controls were functioning perfectly, and which thus cannot be considered as accidental, is a relative lowering of the germination capacity during the period February–March. It appears that a partial secondary dormancy sets in at this period, a possible cause for which is discussed later

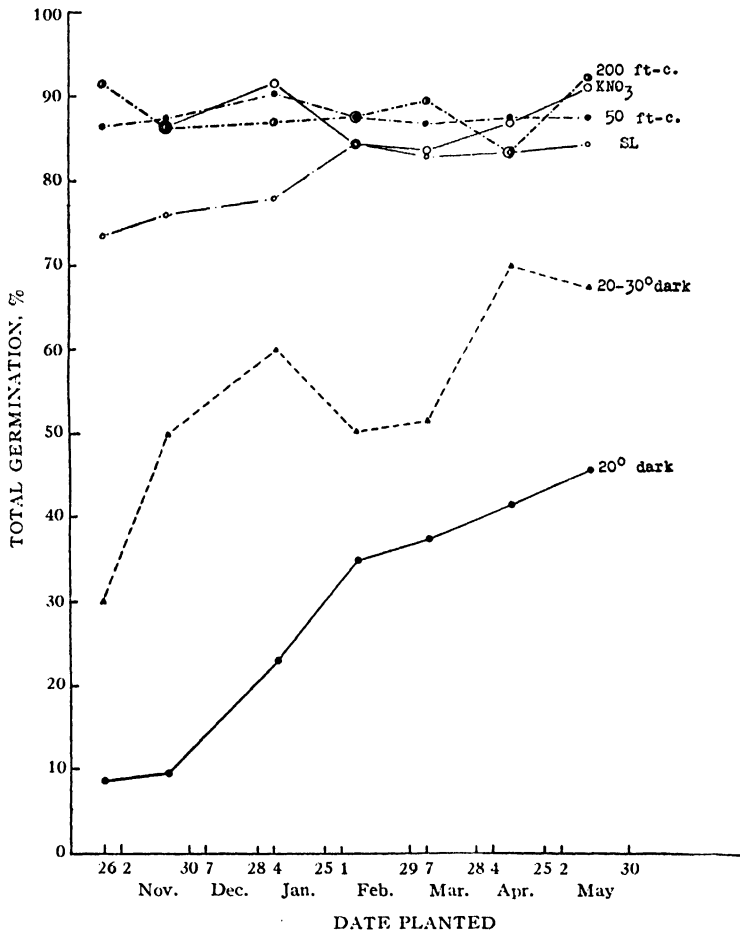


FIG. 3. *A. canina* L. Total germination after 21 days, of tests under various conditions planted at, approximately, monthly intervals. For legend see Fig. 1.

in this paper. Under the most favourable germinating conditions the magnitude of this effect is not great enough to be of serious concern to the seed analyst.

It will be seen from later discussions that 'after-ripening' is a relative term but if for the moment we consider that after-ripening has been completed when the tests given some degree of light have reached their maximum germination, then it may be said that the process lasts until about the turn of the year. Since the tests in subdued light nearly attain the figures given by those with adequate light and, in the case of Brown Top, actually do so,

the three samples may be said to be moderately *low-light-requiring*, while the fact that such a marked depression of germination capacity occurs with complete exclusion of light indicates that they are *highly light-sensitive*. It was unfortunately not possible, on this occasion, to obtain samples of different degrees of maturity; the picture might be different with less mature seed. However, it may be said that their maturity was such as would usually be found in seed going through the trade. It is possible, therefore, that intensity of light may not have had as important a role in the germination of *Agrostis* seeds as has been suggested, even though some light is essential. The conditions of 'subdued light' are of an intensity far lower than could possibly be found in any seed germination laboratory where the seed was given a test in light, which is the standard method for the genus. While the investigations discussed have not served to reveal the lower limit of light intensity at which the maximum germination capacity of after-ripened samples is reached it can be said that an adequate intensity may be provided by placing a 100-watt incandescent lamp, three feet above the level of the seeds placed in a Jacobsen apparatus. Seeds that are in the germination-unripe condition may require the extreme temperature fluctuation suggested by Gadd but time and germinator space did not permit extending the scope of this investigation.

The fact that Hope (4) obtained such a marked difference in germination between seeds placed near a bright window and those placed at the back of the room may have been due to a differential effect of light under the conditions of temperature control available to him. Where conditions are otherwise relatively less favourable, the effect of light may be to counterbalance other (unfavourable) influences, as has been found by the author for lettuce and bluegrass. In the present investigation a sharp alternation of temperature, accurately controlled, has been given.

The aluminium cup method used by Hope to which reference has been made earlier in this paper has the effect of providing a plentiful supply of moisture for the seed without preventing free access of air. The same result is accomplished by the sand tests as used in this investigation and this probably accounts for the somewhat better results obtained by this method than by the other two.

## Part 2. Germination Speed

Because there were great differences between the absolute germination values by different methods and because the speed of germination for the purpose of this study is the proportion of the total germination that occurred early in the test, the actual germination figures were first transformed into the percentage, at each count, of the total germination by the method at the date in question.

For example, the transformation for creeping bent grass planted on the 16th October, 1939, using nitrate, is as follows:—

Days	4	7	10	14	21
Germination, %	12.5	72.0	79.5	80.5	82.5
Speed, %	15	87	96	98	100

As a comparative measure of germination speed the test giving the highest average germination speed was picked out and rated 100%. Each of the other tests was compared with it by finding the deviation from it at each count, summing, dividing by the number of counts and subtracting the result from 100. Since there were considerable differences between the media in the earlier counts of each test, only the sand tests, which in general were superior, have been used in the speed of germination comparisons.

The data so obtained are shown in Figs. 4, 5, and 6 for creeping bent grass, brown top, and velvet bent grass, respectively.

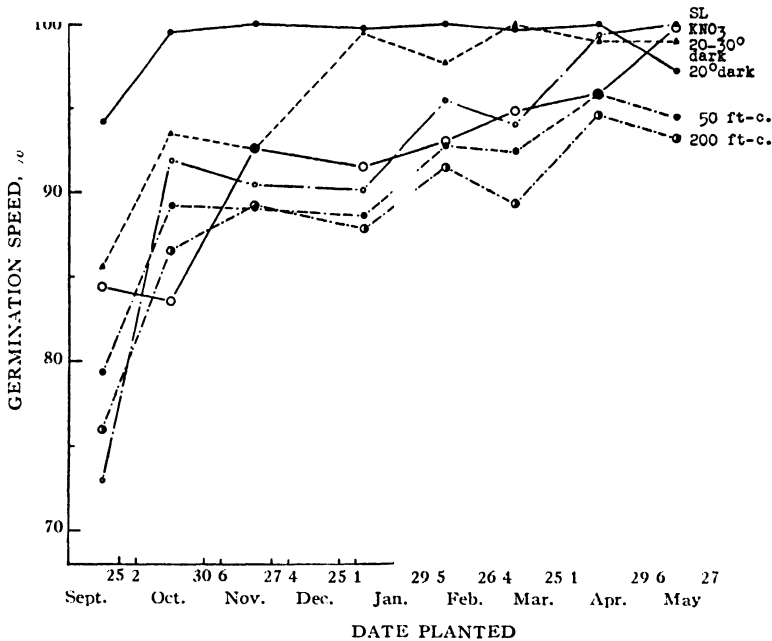


FIG. 4. *A. stolonifera* L. var. *compacta* Hartm. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.

### Discussion, Part 2

In general there is evident an increase of germination speed with time or with the progress of after-ripening, as would be expected. The curves are steep at first, gradually becoming less so, but not always reaching their possible maxima. It should be borne in mind that the data are relative and that it is therefore possible that even the method giving the highest speed and so rated at 100% might have given an even higher speed if the tests could have been continued during succeeding months.

The curves show another feature in common, with one or two exceptions, namely, a sharp rise in February followed by a subsequent depression before the rise occurs again. This should be noted in connection with the dip in the curves for total germination at the same date previously referred to, and will be discussed later.



The February rise is not shared by the nitrate tests except in the doubtful case of Velvet bent grass where the form of the curve appears to be a little exaggerated. Even in this case the rise is not very great nor the subsequent depression very pronounced.

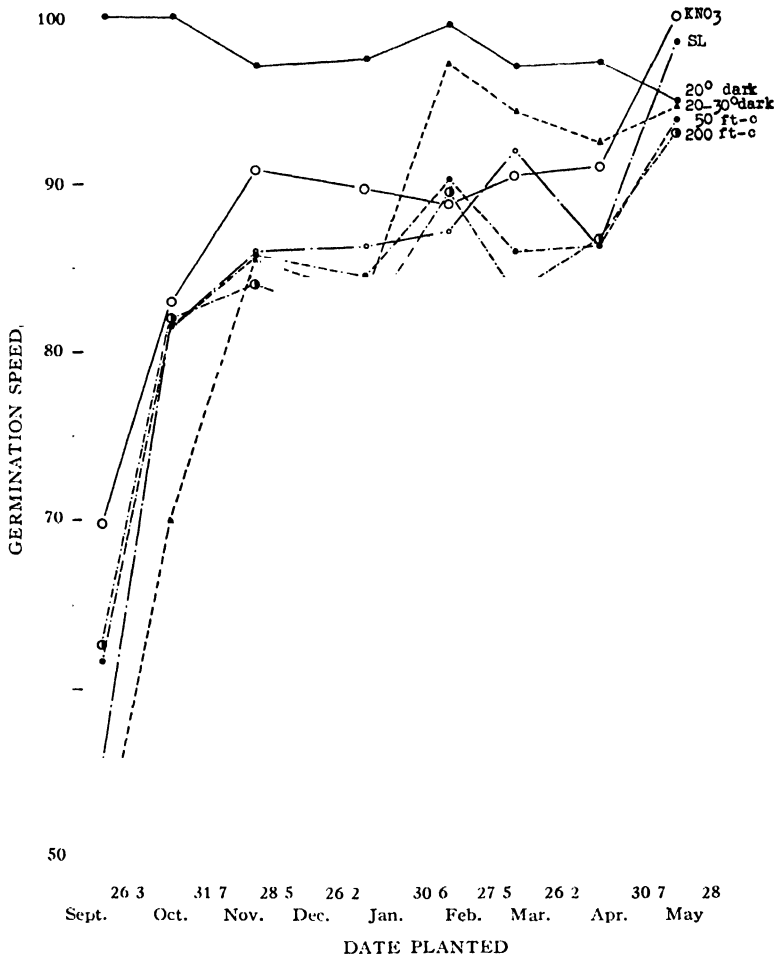


FIG. 5. *A. tenuis* Sibth. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.

### Significance of Data

The curves for 20° C. constant temperature in total absence of light do not share in the tendency towards an upward drift with time. They maintain a fairly consistent and high level, close to 100%. That is, all the germination that is going to occur does so early in the test. (It should be mentioned here that the four-day count was not made on the completely dark tests, in order that the whole of the (presumed) most light-sensitive period of the test might be carried through in complete darkness. Thus the earliest count given was

at seven days. These curves are therefore not strictly comparable to those of tests in which any light experience was given.) The conclusion may be drawn from this that there is a certain proportion of seeds at any date that are germinating-ripe at low temperature in the absence of light, that they

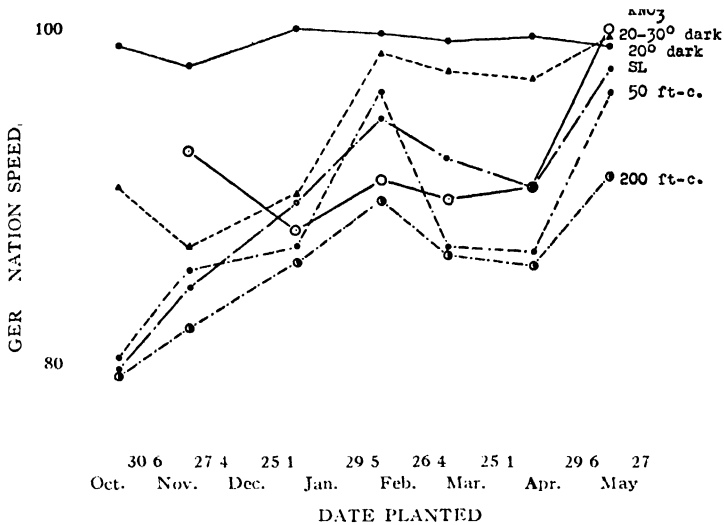


FIG. 6. *A. canina* L. Germination speed of tests planted at, approximately, monthly intervals under various conditions. For legend see Fig. 1.

germinate promptly, and that the remainder remain completely dormant. The cleavage between the two kinds of seeds is surprisingly sharp and rather striking. That the exposure to light that occurs during the counts made at 7, 10, and 14 days is not responsible for this, on account of a possible inhibiting effect of light at the constant low temperature, was proved by duplicate tests given only one count, at 21 days, which did not differ significantly from those given the intermediate counts.

The proportion of such germinating-ripe seeds increases, of course, with time (cf. Figs. 1, 2, and 3). For purposes of reference these will be referred to as Class A.

The curves of germination speed for 20° to 30° C. temperature alternation in complete absence of light do share in the general upward drift with time. The corresponding curves for total germination, however, are at a somewhat higher level generally than those of the 20° C. constant temperature. The difference may be interpreted as a proportion of seeds for which light is not necessary but that may be brought to germinate by a temperature alternation. This proportion will be called Class B. The higher initial germination at 20° to 30° C. indicates that some Class B seeds are brought to germinate quickly but the lower germination speed values show that it requires time to bring some of them to germination.

In order to attempt to present a picture of what happens during the process of after-ripening, the species may be considered separately.

### *Creeping Bent Grass (Fig. 4)*

In this case the initial germination speed at 20° to 30° C. in the dark is fairly high, nearly 86%. It increased rapidly until by January it was practically equal to that at 20° constant and remained so until the end. There is a difference of 17% in total germination, however, in favour of the alternation (Fig. 1), a difference that is substantially maintained except for the reading in May when breakdown of temperature control rendered some of the results uncertain. There is therefore, from January on, a proportion of seeds, 15 to 17%, which are Class *B* but which are as prompt as Class *A* to germinate. There is here, then, an after-ripening change in Class *B* seeds as the season progresses.

Considering now the tests at a temperature alternation in light, it is seen that the germination speed curves run closely parallel and that they are spaced *inversely as the intensity of light*. Reference to Fig. 1 shows that the total germination on the whole is higher with greater light intensity, except that in five readings out of eight, 50 ft-c. is more favourable than 200 ft-c. These observations as to speed and total germination indicate that light tends to depress germination speed and that the higher intensity of light may tend to inhibit, slightly, the germination process.

As Classes *A* and *B* have been designated for convenience in reference, so we may designate a Class *C*, which is brought to germination under the influence of light and temperature alternation and a Class *D*, which does not germinate even under the influence of light. Since 100% germination was never attained with this material, an unknown number of Class *D* may be dead. Class *D*, of fully dormant seeds, is the ultimate source of supply for Classes *A*, *B*, and *C*.

The 'February dip' in the total germination curves in conjunction with the corresponding rise in the speed curves appears to be of some significance. The dip is barely in evidence in the curves for 20° dark and for 50 ft-c. and 200 ft-c., while the dark and subdued light tests at alternating temperature, including nitrate, show the dip clearly. This suggests that it is not Class *A* that is affected nor Class *C* but Class *B*. The higher relative germination speed is not simply the reflection of a lower total germination; a significantly greater number of sprouts grew by the first count than in either the preceding or succeeding tests and the rise in speed is shown by tests that gave little or no dip. This suggests that at this stage of the after-ripening process the normal course would have been that of increased speed of germination and of increased total germination where possible, but that in absence of sufficient light a partial secondary dormancy supervened after the germination process had started—possibly owing to accumulation of respiratory products formed in greater quantity than normal as a result of the greater germination speed coupled with lack of full development of the mechanism for neutralizing the effect of these respiratory products among a proportion of seeds just having passed into Class *B*.

### *Influence of Nitrate*

That Class *B* seeds are not influenced by nitrate is suggested by the fact that the nitrate tests share in the February dip as do the other tests at 20° to 30° C., in subdued light or complete darkness, and Class *B* seeds appear to be responsible for the dip; that Class *D* seeds are so influenced is shown by the fact that higher germination is obtained with the nitrate early in the series than by tests in light; that Class *C* seeds are influenced appears from the fact that the subdued light tests and tests identical except for the use of nitrate run closely parallel after December (Fig. 1) though separated by about 5% in favour of the nitrate.

These observations are not true for *A. tenuis*, which suggests that a number of borderline *B* class seeds exist at this time in this species. This is borne out by the sudden and great increase in Class *B*. Such borderline seeds might still be affected by nitrate, thus lowering the effective number of seeds "Class *B* with respect to nitrate." In this case a nitrate 'dip' would not necessarily be expected. Note also that *A. tenuis* is *lower light-requiring* than the other species so that the subdued light to which the nitrate tests were exposed is relatively more effective.

The fact that the nitrate tests do not show an increase of germination speed but may even show a somewhat lower speed than would otherwise be normal for nitrate tests at this time suggests that a secondary dormancy is induced among a proportion of Class *C* seeds, which may be overcome by light, possibly as a result of an increase of respiration rate brought about by the nitrate.

Nitrate, while serving in the practical germination test as a partial substitute for light, appears to have an entirely different physiological effect.

### *Brown Top (Fig. 5) and Velvet Bent Grass (Fig. 6)*

The picture is not significantly different for these two species except as mentioned above for brown top, though the argument has been developed from the results for creeping bent grass alone because they seemed a little more clear-cut. Fig. 5 compares fairly closely with Fig. 4, the chief differences being that the curve for 20° to 30° C. in the dark starts at a much lower point in the case of brown top and lags considerably in comparison with that for creeping bent until February, when it shows a peak in contrast to a dip. At the first date the relative positions of the curves of the tests given some light are the reverse of what they are at the last. The 20° C. constant curve shows a marked peak in February. In this curve also there is a tendency towards a slight fall in germination speed with time, the significance of which is questionable. The curves of Fig. 5 also show a rather stronger tendency towards a pre-February depression while the peak for the curve of 20° to 30° C. in subdued light occurs in March rather than February. In the case of velvet bent grass, Fig. 6, there are no results for September nor in October for the

nitrate tests, so that the earliest stages of after-ripening cannot be compared. However, the curves when examined in detail do not present a greatly different picture from those of the other two species, the chief differences being a somewhat more strongly marked post-February depression of germination speed and, for the test at 50 ft.-c., a relatively greater February peak. All these differences can be ascribed to differences of degree or date rather than of kind and the general picture remains unaltered.

The data are too voluminous to present in detail, but specimen data on final counts on *A. tenuis* are presented in Table I.

TABLE I

GERMINATION OF *Agrostis tenuis* SIRTH. FROM SEPTEMBER, 1939, TO MAY, 1940. TOTAL PERCENTAGE GERMINATION IN 21 DAYS, BY MONTHS

Medium	Temp.	Light	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Sand	20° C.	None	2.0	4.5	17.5	—	35.5	39.5	44.0	48.0	61.0
Kimpak			0.0	6.5	13.0	—	26.5	37.5	42.0	45.0	59.1
Filter paper			0.5	2.0	14.0	—	35.5	44.0	41.5	61.5	67.5
Sand	20° to 30° C.	None	2.0	22.5	51.5	—	72.0**	58.0	66.0	80.0	80.0
Kimpak			3.5	23.0	41.0	—	74.0**	55.0	67.0	70.0	83.5
Filter paper			2.5	23.5	51.0	—	87.0**	55.0	69.5	79.6	80.5
Sand	20° to 30° C.	SL*	50.0	76.5	90.8	—	92.5	88.0	94.0	91.0	91.0
Kimpak			34.5	79.5	83.0	—	90.0	93.0	89.0	91.5	91.0
Filter paper			45.0	79.0	90.5	—	90.0	93.5	89.5	95.5	93.5
Sand	20° to 30° C.	50 ft.-c.	85.5	91.0	93.5	—	96.0	96.0	89.5	—	—
Kimpak			81.5	92.5	93.5	—	90.0	91.0	94.5	—	—
Filter paper			79.0	88.0	90.0	—	94.0	94.0	96.5	—	—
Sand	20° to 30° C.	200 ft.-c.	90.0	90.5	90.5	—	92.0	90.5	90.5	95.5	92.5
Kimpak			87.5	91.5	95.0	—	92.0	91.0	95.5	92.0	92.5
Filter paper			91.0	91.0	94.5	—	94.0	91.0	96.0	93.5	94.0
Sand	20° to 30° C.	SL and KNO <sub>3</sub>	86.0	93.5	93.5	—	94.5	91.0	95.0	91.0	94.0
Kimpak			—	91.0	94.5	—	95.0	93.5	90.5	93.0	95.0
Filter paper			88.0	92.5	90.5	—	94.0	96.5	92.0	93.0	95.0
Sand	20° to 30° C.	50 ft.-c. fluorescent light	—	92.0	93.0	—	94.5	88.0	94.5	95.5	95.5
Kimpak			—	93.0	91.0	—	93.5	92.5	93.0	89.5	95.5
Filter paper			—	84.5	94.0	—	92.5	91.0	93.5	93.5	92.5

\* SL = Subdued light.

\*\* Tests left at 30° C. during night of 10/1/40 in error.

Note: All tests were affected in May on the 7th, 8th, and 15th days after planting by breakdown of temperature control. Temperatures in the morning were found to be about 30° C.

### Acknowledgment

My thanks are due to my assistant, Miss A. W. Anderson, for her painstaking care in planting and counting the tests and to Dr. H. T. Güssow, formerly Dominion Botanist, and Dr. H. B. Sifton, Department of Botany, University of Toronto, for a critical reading of the manuscript.

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# Canadian Journal of Research

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## THE EFFECT OF DDT ON THE STEM RUST REACTION OF KHAPLI WHEAT<sup>1</sup>

BY T. JOHNSON<sup>2</sup>

### Abstract

Seedling leaves of Khapli wheat—a variety highly resistant to physiologic races of wheat stem rust prevalent in North America—became susceptible to stem rust a few days after they had been sprayed with DDT (1 oz. in 5 gal. water). The response to DDT was highly specific, as only one other of the resistant wheats tested (Arnautka) showed any indication of susceptibility consequent on spraying. Specificity of response was also indicated by the development of marked chlorosis on leaves of some varieties, whereas other varieties remained unaffected.

DDT (dichlorodiphenyltrichloroethane) has recently come into use for the control of white flies and other insects in the greenhouses of the Dominion Laboratory of Plant Pathology, Winnipeg, Man. Among the plants sprayed in one of the applications were certain wheat varieties employed as differential hosts for physiologic races of stem rust of wheat (*Puccinia graminis Tritici* Erikss. & Henn.). The wheat varieties had been inoculated, in the seedling stage, with race 17 of this rust four days prior to spraying with DDT. When observations were made on rust development, 10 days after the spraying took place, it was noted that the rust pustules (uredia) on the highly resistant variety Khapli (*T. dicoccum*) were somewhat larger than the minute type 1 pustule normally produced on Khapli by race 17 and, indeed, by all other races of stem rust commonly prevalent in North America. The plants were kept for further observation and it was noted that the rust pustules on Khapli continued to increase in size until, 24 days after spraying took place, they were classified as type 4—a type of pustule associated with full susceptibility of the host.

To determine whether or not the pathogenicity of the rust had changed, spores of these large pustules were sown on the leaves of several wheat varieties including Khapli. The pustules that later developed on these varieties were typical of race 17, those on Khapli being classified as the characteristic type 1 pustule formed on that variety. Further observation showed no subsequent increase in the size of these pustules. It was therefore evident that the DDT had effected no change in the pathogenicity of the rust.

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Contribution No. 842 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

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Subsequently, an experiment was made to determine the effect of DDT on infections caused by other races of wheat stem rust. Two sets of differential hosts (wheat varieties) were inoculated with races 15 and 56, respectively, on November 7. Three days later one set inoculated with each of these races was sprayed with a solution of DDT of the same concentration as that previously used for white fly control (1 oz. DDT in 1 litre of 95% ethyl alcohol added to 5 gal. water containing a small amount of Aerosol OT). The plants were sprayed as uniformly as possible to ensure that all varieties received about the same application of DDT. A control set, for each race, was sprayed with exactly the same solution except that no DDT was present.

When observations were made on rust development, nine days after the spray was applied, both races had produced considerably larger rust pustules on the Khapli plants sprayed with DDT than on those sprayed with the control solution. The pustules on the treated Khapli plants were recorded as type 3, for race 56, and 3—, for race 15, whereas those on the control plants were in both cases recorded as type 1—. Infections on treated and control plants of Khapli are shown in Fig. 1 as they appeared 13 days after spraying.

Examination of the infection types present on the other differential hosts made it clear that response to DDT is highly specific in that the presence or absence of any response depends on the host variety infected. Among the differential hosts were four varieties that possess about the same degree of resistance as Khapli to race 56, namely, Arnautka, Mindum, Spelmars, and Einkorn. Only on Arnautka was there any indication of larger pustules on the plants treated with DDT than on the control plants.

It was evident too, from examination of the treated plants, that some wheat varieties show more tolerance towards DDT than others. Leaves of the varieties Arnautka, Spelmars, and Khapli developed a pronounced chlorosis in about a week after they were sprayed, whereas the varieties Kota, Mindum, and Einkorn showed no indication of chlorosis. Faint chlorosis was noted on the varieties Marquis, Kanred, Kubanka, Acme, and Vernal. As only one concentration of DDT was used in the experiments under consideration, the possibility exists that more concentrated solutions might produce a high degree of chlorosis on all varieties.

On treated Khapli leaves there appeared to be a marked relationship between chlorosis and rust development. Chlorosis progressed gradually until, in some leaves, green colour was no longer visible. The virtual disappearance of green pigment, however, did not inhibit the increase in size of the rust pustules, which grew gradually larger until the leaves withered.

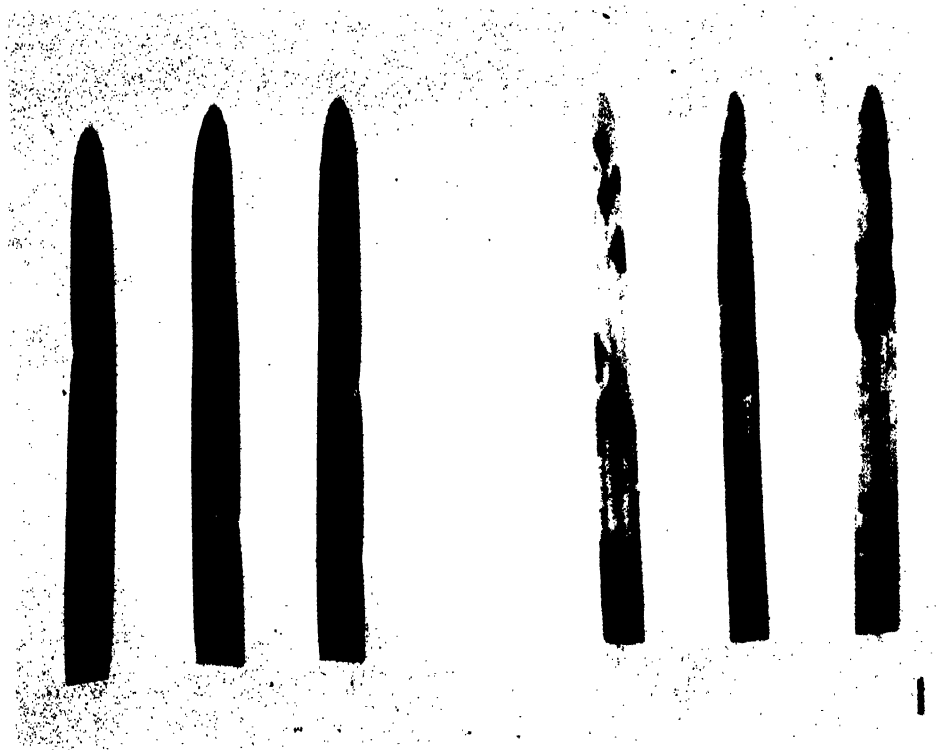
#### EXPLANATION OF FIGURE

FIG. 1. *The effect of DDT on stem rust reaction of seedling leaves of Khapli wheat, 16 days after inoculation and 13 days after spraying with DDT.*

*Left: Leaves sprayed with control solution.*

*Right: Leaves sprayed with DDT.*

PLATE I





The immediate significance of the foregoing observations lies in the fact that the possible effect of DDT on host reactions should not be overlooked when this substance is being used for insect control. Of more fundamental import is the possibility that the influence of DDT on host reaction may open up a new approach to the study of the nature of rust resistance. In this connection, the following facts seem suggestive. The two varieties Khapli and Spelmars normally show about the same degree of resistance to race 56 and, when sprayed with DDT they developed about the same amount of chlorosis. Yet Khapli became susceptible when sprayed with DDT while Spelmars did not. If, as seems likely, the general physiological effect of DDT is the same on both varieties, it seems reasonable to assume that the physiological basis of rust resistance differs either quantitatively or qualitatively in the two varieties. In any event, the observations recorded above suggest that it would be profitable to study the physiological effects of DDT on wheat varieties and to correlate these effects with rust reaction.

## PHYSIOLOGIC RACES OF *PUCCINIA GRAMINIS TRITICI* IN CANADA, 1919 TO 1944<sup>1</sup>

BY MARGARET NEWTON AND T. JOHNSON<sup>2</sup>

### Abstract

During the period 1919 to 1944, 65 physiologic races were identified from a total of 4543 isolates derived from uredial collections of *Puccinia graminis* Pers. var. *tritici* Erikss. & Henn. Forty-nine races were obtained in the Prairie Provinces from a study of 3475 isolates; 40 in Eastern Canada from 1013 isolates, and 12 in British Columbia from 55 isolates. During this period, the predominant races have shown notable fluctuations in their prevalence. Races 36, 17, and 21 were the most common races until shortly after 1930 and were largely responsible for the severe rust losses suffered by Marquis and other common wheats during the decade preceding that year. Races 34 and 49 were collected frequently from 1927 to about 1935. All of these races diminished greatly in their prevalence between 1930 and 1936, whereas race 56, which was first collected in Canada in 1931, has become the predominant race since 1934. It was this race that played a major part in the stem rust epiphytotic of 1935. Another recent change in the racial population was a recrudescence in 1940 of race 17, which for several previous years had been of minor importance. In 1941 this race challenged the pre-eminent position of race 56 but receded again in succeeding years to minor significance.

The distribution of races is somewhat similar but not identical in different parts of Canada. Races 36 and 21 have been relatively more common in the Prairie Provinces than in Eastern Canada, while the contrary is true of race 38. Only about a dozen of the 65 races collected in Canada have thus far assumed much economic importance, a few others may be considered of minor significance, but at least two-thirds of the races have been found only occasionally and have, for reasons not fully understood, failed to gain even a limited distribution.

A comparison of the number of physiologic races collected in Eastern Canada and the Prairie Provinces, respectively, has indicated a somewhat greater variety of physiologic races in the former region, a condition that may perhaps be explained by the presence of the common barberry in many localities in Eastern Canada.

### Introduction

Specialization in *Puccinia graminis* Pers. var. *tritici* Erikss. & Henn. was first reported by Stakman and Piemeisel in 1917 (21, 22). The first investigations on the specialization of this rust in Canada were undertaken by the senior author in 1918 at the suggestion and with the advice of the late Professor W. P. Fraser, and were continued by her until April, 1922 (9, 10). From 1922 to 1924 studies on physiologic races of wheat stem rust in the Prairie Provinces were continued by Professor Fraser (1, p. 45; 2, pp. 40-41; 3, p. 68; 15, p. 100). From 1925 until the present time, surveys of the prevalence of physiologic races have been carried out annually by the present writers. The work done during the period 1919 to 1930 was fully reported in 1932 (12) and later a brief account was given of the races isolated up to 1936 (11). As work carried out subsequent to 1936 has never been placed on

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record and as, moreover, records are now available on the distribution of physiologic races in the Prairie Provinces of Canada for a period of more than a quarter of a century, it seemed desirable to bring together in one publication the information now on hand.

During the seven years, from 1919 to 1925, the study of the distribution of physiologic races was almost entirely confined to the three Prairie Provinces of Western Canada. From that time onward attempts were made to extend the survey of physiologic races to other parts of Canada with the result that, in most years, collections from all the provinces have been studied.

### Procedure and Methods

In securing rust collections the writers are indebted to numerous individuals and institutions throughout Canada. In the three Prairie Provinces, surveys have been conducted annually by the staffs of the laboratories of plant pathology at Winnipeg, Man., Saskatoon, Sask., and Edmonton, Alta. Rust collections from other parts of the country have been made chiefly by individuals connected with the various laboratories of plant pathology and agricultural colleges. Collections from the chief cereal growing areas of Canada were facilitated by the maintenance of uniform rust nursery plots of cereal varieties at experimental farms and stations in these areas during the periods 1920 to 1929 and 1936 to 1944. During the earlier period, while the nurseries were relatively few, annual visits were made to them. During the latter period, when the nurseries became numerous and widely distributed and visits could no longer be made to them, small sheaves of each of the varieties grown were shipped to the Winnipeg laboratory for examination.

Methods of inoculation with urediospore collections and the procedure followed in the identification of physiologic races have been described in detail elsewhere (12, 18).

During most of the period covered by the surveys, isolation of physiologic races from aeciospores has been very limited. Very few of the earlier attempts to establish cultures from aeciospores were successful, chiefly because the aecia had to be shipped long distances and methods of inoculation suitable to aeciospores in a more or less dried-out condition had not been worked out. In more recent years, however, a simple and relatively satisfactory method of inoculation has been devised. Briefly, this consists in cutting out individual aecial pustules or leaf areas bearing numerous aecia and pressing these firmly against a wet blotting paper in a Petri dish cover, with the aecia facing outward, until the leaf sections adhere to the paper. The aecia are then sprayed with a fine spray from an atomizer and suspended above a glass slide in a Petri dish bottom over night. On the following morning it is usually found that large numbers of aeciospores have been discharged on the glass slide. The aeciospores so discharged are applied to seedling cereal leaves by drawing the slide gently over the leaf surfaces. If any appreciable germination of the spores takes place, a reasonably good infection is ensured.

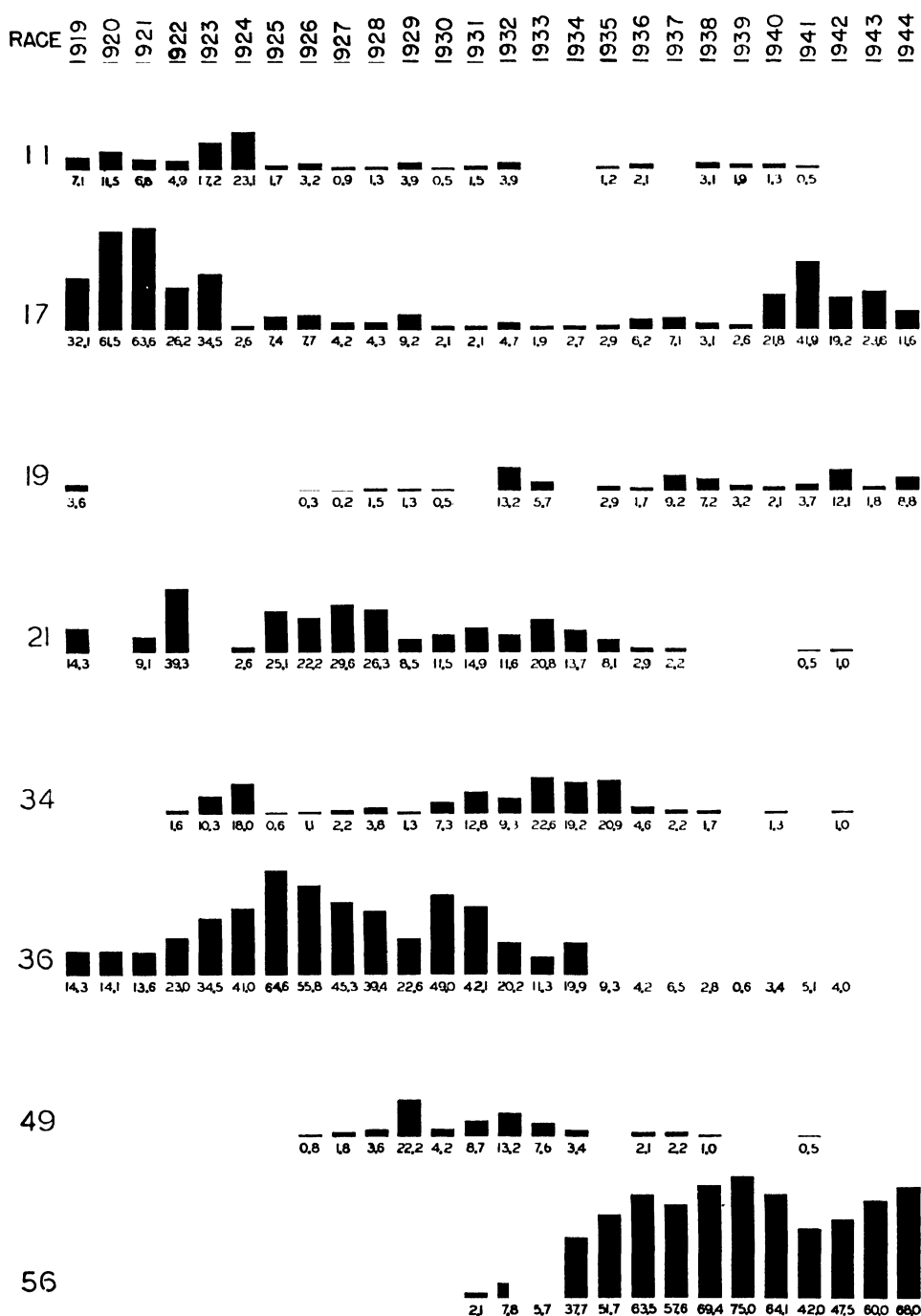


FIG. 1. Annual frequency of occurrence of the more important physiologic races of wheat stem rust isolated in Canada during the period 1919 to 1944, expressed in per cent of the total isolates for each year. In the above chart, certain similar races recorded separately in Table I, are recorded as one race, i.e., race 32 is included with race 11, race 29 with race 17, races 3 and 18 with race 36, and race 125 with race 56.

## Results of the Surveys

The annual prevalence of physiologic races is shown in Table I and that of the more important physiologic races is represented diagrammatically in Fig. 1. It is clear that, when surveys were first undertaken in 1919, and for several years thereafter, there were two predominant groups of physiologic races. One of these groups is composed of races 3, 18, and 36, which, in the earlier years, were recorded as distinct races, but were subsequently classified as a single race (race 36) owing to their close similarity to each other. The other group consists of races 17 and 29, which, for the same reason, were grouped together as race 17. Another race of considerable importance in those early years was race 21. All of these races were characterized by the ability to attack heavily the hard red spring wheats then grown in Canada and the north central United States, namely, varieties such as Marquis, Red Fife, Preston, Ruby, Haynes Bluestem, and others. It was undoubtedly these races that were chiefly responsible for the recurrent damage to hard red spring wheat—damage so severe that farmers in certain areas of Manitoba and North Dakota were forced to abandon these wheats in favour of the more rust resistant durum wheat.

Races 36, 17, and 21 maintained their predominance until the early thirties but as early as the late twenties other races were assuming importance. Races 34 and 49 began to be common about 1927 and remained so until about 1935. Race 38 has been one of the common races, especially in Eastern Canada, since 1926.

Between 1930 and 1936 notable changes took place in the prevalence of the common races. During this period, races 17, 21, 34, 36, and 49 were found less and less frequently, whereas race 56, which was first collected in the field in Canada in 1931, had become three years later the predominant race, a position it has maintained up to the present time. Since the rise of race 56 to pre-eminence, the only pronounced change in racial population was the sudden increase, in 1940, in the prevalence of race 17 which, in 1941, challenged the predominant position of race 56. Subsequent surveys, however, have shown a sharp decrease in the prevalence of race 17.

The above outline, though based largely on the results of surveys in the Prairie Provinces, is also, in general, applicable to other parts of the country. Races that are important over a period of years in the Prairie region are also common elsewhere in Canada during the same period. No absolute agreement, however, exists between the predominance of certain races in the Prairie Provinces and Eastern Canada. For example, during the period 1926 to 1944, race 36, which was the most commonly collected race in the Prairie region, was in third place in the Maritime Provinces and in Ontario and Quebec, and race 21, which was in third place in the Prairie region, took only fifth place in the eastern regions. Still more striking is the relative prevalence of race 38 in the east and the west. This race, which was in second place in both of



TABLE I

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
Maritime Provinces																											
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
11	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2
14	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
15	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
17	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	7
19	-	-	-	-	-	-	-	1	1	1	2	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	13
21	-	-	-	-	-	-	-	13	3	6	3	1	-	-	-	-	-	-	3	-	-	-	-	-	-	-	29
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
29	-	-	-	-	-	-	-	2	-	-	2	-	-	-	-	-	-	3	4	7	-	9	6	1	2	-	36
30	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
32	-	-	-	-	-	-	-	2	-	-	2	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	8
34	-	-	-	-	-	-	-	-	-	2	-	-	-	-	1	1	-	1	3	2	-	-	-	-	-	-	17
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
36	-	-	-	-	-	-	-	30	6	5	3	4	1	-	-	-	4	-	2	1	-	-	-	-	-	-	56
38	-	-	-	-	-	-	-	6	9	13	10	4	1	-	5	-	5	7	7	11	3	7	-	3	2	-	93
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	1	-	-	-	1	-	-	6
49	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	2	2	-	-	-	-	-	-	8
50	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	10	14	24	61	21	16	4	1	4	5	162
64	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
74	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
75	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	2
183	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1

TABLE 1—Continued

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—Continued

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
1	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
11	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3
14	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
17	-	-	-	-	-	-	-	-	2	3	2	-	-	-	-	-	-	1	-	1	-	1	-	-	-	-	11
19	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	18
21	-	-	-	-	-	-	2	10	11	3	4	1	3	2	2	-	2	1	1	1	-	-	2	-	2	4	43
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
29	-	-	-	-	-	-	-	7	-	2	-	-	-	-	-	-	-	4	-	-	1	2	9	3	3	4	54
30	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
32	-	-	-	-	-	-	-	4	2	-	3	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	11
33	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
34	-	-	-	-	-	-	-	-	1	2	1	-	2	-	-	-	5	1	-	-	-	-	3	-	-	-	17
36	-	-	-	-	-	-	4	18	13	9	1	6	3	3	1	-	2	3	2	-	-	-	-	1	-	-	66
38	-	-	-	-	-	-	-	11	4	10	19	8	1	2	1	-	13	2	2	6	5	1	4	7	6	1	101
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	1	1	-	-	-	-	-	-	6
48	-	-	-	-	-	-	-	1	-	-	1	1	1	1	1	-	-	-	-	-	-	-	1	-	-	-	3
49	-	-	-	-	-	-	-	-	1	3	1	1	1	1	1	-	-	-	1	-	-	-	-	-	-	-	10
50	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
53	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	35	20	27	14	19	15	5	15	17	179
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	3
63	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
78	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
84	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
89	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Ontario and Quebec

TABLE I—Continued

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—Continued

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
Ontario and Quebec—Concluded																											
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
113	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	2
125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	4
192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Prairie Provinces																											
1	2	1	-	-	-	-	-	-	-	2	10	1	2	2	-	1	-	-	-	-	-	-	-	-	-	-	21
2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
3	-	4	3	10	10	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	45
9	4	6	2	3	-	-	-	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	21
10	-	-	-	-	-	-	-	-	-	-	-	-	3	4	-	-	-	-	-	-	-	-	-	-	-	-	7
11	2	5	2	3	5	9	-	-	-	3	2	-	1	3	-	-	1	-	-	-	-	1	-	-	-	-	37
12	-	2	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
14	-	-	-	-	-	-	-	1	15	1	4	3	3	2	4	-	-	-	-	-	-	-	-	-	-	-	34
15	1	-	-	-	-	-	-	2	8	16	-	-	-	1	-	-	-	-	-	1	2	-	-	1	-	-	33
16	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
17	9	31	27	16	10	1	-	1	13	6	9	-	3	6	-	2	-	3	4	-	-	2	-	-	3	6	152
18	4	7	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
19	1	-	-	-	-	-	-	-	-	5	1	1	-	17	3	-	5	4	11	15	4	5	5	10	-	8	95
21	4	-	4	24	-	1	42	61	118	93	19	19	23	13	9	20	9	6	3	-	-	-	1	-	-	-	469
23	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
24	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
27	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
29	-	17	1	-	-	-	13	19	4	9	13	4	1	-	1	2	4	4	3	1	2	31	65	14	15	3	226
30	-	1	-	-	-	-	1	7	5	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	16
32	-	4	1	-	-	-	3	6	2	-	5	1	-	2	-	-	1	3	-	3	2	2	1	-	-	-	36

TABLE I—Continued

D: DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—Continued

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
Prairie Provinces—Concluded																											
33	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
34	-	-	-	-	-	-	-	-	-	-	-	-	23	12	11	27	24	9	1	3	-	-	-	-	-	-	162
36	-	-	-	-	-	-	-	84	139	65	36	84	75	23	5	29	10	4	8	6	6	-	10	3	-	-	926
38	-	-	-	-	-	-	-	27	9	13	36	27	8	5	3	3	-	3	4	4	4	8	1	-	1	10	137
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	2	2	2	-	-	-	-	5	15
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
49	-	-	-	-	-	-	-	6	11	65	1	6	16	16	3	5	-	5	1	1	-	-	1	-	-	-	139
50	-	-	-	-	-	-	-	-	-	5	-	-	2	1	2	-	-	-	-	-	-	-	-	-	-	-	13
51	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
52	-	-	-	-	-	-	-	-	6	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	11
53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
56	-	-	-	-	-	-	-	-	-	-	-	-	-	10	2	41	64	98	54	112	80	105	64	40	45	68	786
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
81	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
86	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	2
87	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
97	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
123	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	39
125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	3	3	7	-	1	3	-	-	-	9	1
136	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	2
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	1
177	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
179	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1

TABLE 1—*Concluded*

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis* VAR. *Tritici* COLLECTED ON CEREALS AND GRASSES FROM 1919 TO 1944 (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)—*Concluded*

Race	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Total
British Columbia																											
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
14	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4
21	-	-	-	-	-	-	-	-	1	1	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	6
23	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
36	-	-	-	-	-	-	-	1	-	-	-	-	3	-	-	-	-	3	-	1	-	1	-	-	-	-	9
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
56	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	1	6	6	1	2	1	20
61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
78	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
113	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	3	-	5
Total	28	78	44	61	29	39	175	378	450	391	306	192	195	129	53	146	172	241	184	291	156	234	215	99	110	147	4543

the eastern regions, took only seventh place in the Prairie Provinces. The relatively lower incidence of this race in the Prairie Provinces is perhaps due to the fact that Marquis wheat, which is resistant to it, was for many years the prevailing wheat variety in that region.

The introduction of new wheat varieties undoubtedly had some influence on the prevalence of individual races. It has been pointed out (6, 16, 19, 20) that Ceres wheat, which came to be widely grown in the north central United States and in Manitoba from 1926 onwards, is more susceptible to race 56 than to the races prevalent at the time of its introduction; and it has been maintained (19, 20) that the susceptibility of Ceres to this race was a prime factor in the increase in its prevalence.

It cannot be doubted that the more recent introduction of stem rust resistant wheat varieties has greatly diminished the amount of stem rust inoculum in the regions in which they are grown. Whether or not their introduction has effected any change in the relative abundance of the different physiologic races is a more doubtful matter. As most of the new varieties are almost equally resistant to the various races common in North America, there is little likelihood that the new varieties would have the effect of increasing one race in preference to another. It is, however, not at all impossible that some little known or perhaps unknown race may possess ability to attack them and thereby gain greater distribution in Canada in future years.

### The Role of Barberry in the Origination of New Races of Wheat Stem Rust

It has been amply demonstrated in laboratory experiments that physiologic races of wheat stem rust may, through hybridization and selfing on the barberry, produce races pathogenically different from those that were crossed or selfed (5, 8, 13, 14, 17). Undoubtedly, these same processes of crossing and selfing of races occur on naturally infected barberry. As stem rust spreads readily from barberry bushes to wheat and susceptible grasses growing in the vicinity of infected bushes, there is every reason to suppose that a considerable variety of physiologic races is being passed on every year from barberry to susceptible cereals and grasses.

In the Prairie Provinces, in which the great bulk of Canada's wheat crop is grown, barberry is virtually non-existent. In Eastern Canada and British Columbia, where scattered plantings of this shrub exist, there is a possibility that barberry may play a part in the origination of new physiologic races. Wheat, however, is not extensively grown in either of these regions, and the limited infection studies thus far carried out, confined entirely to aeciospores collected in Eastern Canada, indicate that there the rust on barberry is, for the most part, rye stem rust (*Puccinia graminis* var. *Secalis*). Of 33 collections of aecia gathered in 1944 in Ontario, Quebec, and the Maritime Provinces, 30 contained the *Secalis* variety either alone or mixed with other varieties.

The results of this study are summarized below:

<i>Secalis</i> variety alone.....	19	collections
<i>Secalis</i> + <i>Agrostidis</i> varieties.....	6	"
<i>Secalis</i> + <i>Avenae</i> varieties.....	3	"
<i>Secalis</i> + <i>Avenae</i> + <i>Agrostidis</i> varieties.....	1	"
<i>Secalis</i> + <i>Avenae</i> + <i>Tritici</i> varieties.....	1	"
<i>Agrostidis</i> + <i>Tritici</i> varieties.....	1	"
<i>Agrostidis</i> + <i>Poa</i> varieties.....	1	"
<i>Poa</i> variety alone.....	1	"

The predominance of the *Secalis* variety in these collections is probably the result of the ubiquitous presence of *Agropyron repens*, a grass susceptible to rye stem rust. Undoubtedly, aecia of wheat stem rust occur also each year on the barberry and are the means of disseminating that rust to such susceptible grasses or cereals as may be growing in the vicinity. The fact that, as the present studies have shown, a proportionally greater number of races occur in Eastern Canada than in the Prairie Provinces is perhaps a reflection of the role played by barberry in the production and dissemination of physiologic races. In Eastern Canada, 40 races occurred in 1013 isolates or one race for each 25.3 isolates. In the Prairie Provinces, 49 races were identified in 3475 isolates or one race per 70.9 isolates. Of the 40 races collected in Eastern Canada, 15 were not found in the Prairie Provinces. On the other hand, the collections made in the Prairie Provinces contained 24 races that have not been found in Eastern Canada. The collections made in British Columbia are too few to warrant comparison. However, in a total of 55 isolates there occurred three races not found in the adjacent Prairie Provinces.

From the figures given above, it is evident that there are many races that are not common to the Prairie Provinces and the rest of Canada. All of these, however, are races that occur only rarely. The races that are common in one region have always been found more or less commonly in the other regions. How these rare races originate and why they do not become permanent entities of the racial population are unsolved problems. In Eastern Canada and British Columbia, the races that have never been found in the prairie region may possibly be attributable to barberry. Such races could originate through selfing or crossing of wheat stem rust races, or, owing to numerous infections of barberry by rye stem rust, they could arise from crosses between wheat and rye stem rust. Races originating in such inter-varietal crosses would probably, in most cases, be pathogenically weak on wheat (7).

Although most of the physiologic races now established in nature probably arose from barberry at some time or another, there can be little doubt that most new races produced on barberry do not become permanently established on cereals as economically important physiologic races. It may be inferred from the characteristics of many races produced in crossing and selfing studies

that some races originating on barberry are ill adapted for survival. Races produced on barberry differ from each other not only in pathogenicity but also in colour, abundance of urediospore production, and in the length of time elapsing between the commencement of uredial production and the formation of telia. It is almost certain that races with a long period of urediospore production have an advantage over races with a shorter period. Some races originating from barberry are known to have short uredial periods, a fact that would limit their abundance and distribution and would therefore militate against their permanent establishment in nature. The inheritance of this characteristic has not been studied extensively and is therefore not fully understood. Nevertheless, unpublished work by the writers has shown that some races with a brief uredial stage will give rise, when selfed, to races that vary considerably in the lengths of their periods of urediospore production, and may, consequently, after infection of barberry, produce races with uredial periods long enough to give them a good chance of survival. There exists, therefore, always the possibility that races not well adapted for survival may, through the instrumentality of the barberry, produce races that may survive and become important.

A characteristic of many races isolated from barberry, but which is not common in races collected on wheat or grasses, is the ability to attack certain emmer wheats. One such race that has attracted attention in recent years, though it has not yet become common, is race 15, and particularly its biotype 15*B*, which is pathogenic to some of the more recently developed rust resistant wheats (4). The possibility of the barberry playing a role in the origin of such highly pathogenic races should not be overlooked. There is, in fact, some evidence that races with ability to attack emmer wheats may originate in the selfing of races that lack this ability. Selfing studies by the writers with 14 races to which Vernal emmer wheat was highly resistant showed that seven of these races were heterozygous for ability to attack Vernal heavily; that is, they produced in their progeny some races to which Vernal was susceptible. Infection studies with teliospores collected on grasses have revealed similar races. For example, aecia on barberry inoculated with teliospores collected on *Agropyron trachycaulum* (Link) Malte var. *unilaterale* (Cassidy) Malte (*A. subsecundum* (Link) Hitchc.) gave rise to nine races of which four attacked Vernal heavily; and aecia derived from teliospores on *Agropyron trachycaulum* (Link) Malte var. *typicum* Fernald (*A. pauciflorum* (Schwein.) Hitchc.) produced 10 races of which five attacked Vernal moderately or heavily.

For some unknown reason, races that attack Vernal heavily have not as yet become established as important elements in the physiologic race population. In Canada, they are represented by only 108 out of 4543 isolates, or 2.4 % of all isolates. The fact that they are rather common in rust derived from barberry indicates the possibility of their spread and permanent establishment on cereals.



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# OCCURRENCE OF *BACILLUS POLYMYXA* (PRAZ.) MIG. IN ALBERTA SOILS WITH SPECIAL REFERENCE TO ITS PATHOGENICITY ON POTATO TUBERS<sup>1</sup>

BY A. W. JACKSON AND A. W. HENRY<sup>2</sup>

## Abstract

The spore-forming bacterium, *Bacillus polymyxa*, was found to occur widely in Alberta soils. All isolates obtained from the soil, together with several from other sources, proved capable of rotting potato tubers when introduced through wounds under conditions of abundant moisture and high temperature.

In these studies pure cultures of *Bacillus polymyxa* rotted potato slices at temperatures from 20° C. to 45° C. and whole tubers at temperatures from 30° C. to 45° C. but caused no damage below the minimum temperatures mentioned.

Though potato tubers and other vegetables that are formed in the soil probably often come in contact with *Bacillus polymyxa*, they are not likely to be rotted by it except when temperature and other factors are favourable. In general, soil temperatures in the field in Alberta are not conducive to the decay of potatoes by this organism, but those near the soil surface may on occasion reach favourable levels. The fact that *B. polymyxa* can be isolated quite frequently from rotted stored potatoes indicates that it is of some importance as a cause of decay in harvested potatoes. If, however, recommended storage temperatures are maintained in the storage quarters no damage from this organism should occur there.

## Introduction

*Bacillus polymyxa* is a bacterium that is better known for its ability to ferment starch to form chiefly 2,3-butanediol and ethyl alcohol than as a plant pathogen. This organism has received some attention during the war just ended as a means of producing the above compounds, which have been useful in the war effort (7).

The idea that plant pathogenic bacteria are not spore-formers appears to have been quite generally accepted. However, a number of workers have reported certain spore-bearing bacteria to be capable of rotting vegetables and fruits. According to Brierley (4), Lepoutre in 1902 reported *Bacillus mesentericus vulgatus* to be capable of attacking potato tubers and Van Hall found *B. subtilis* and *B. vulgatus* pathogenic to potato tubers and other vegetables when incubated at 37° C. and 42° C. Brierley (4) isolated cultures of *B. mesentericus* and found them to be capable of rotting potato tubers over a wide range of temperature. Cultures used to inoculate the tubers were previously pasteurized at 80° C. for 15 min., thus assuring that they originated from spores. In 1943, Dowson (6) reported the isolation of *B. polymyxa* from a sample of rotting potato. This isolate proved capable of rotting slices of fresh potato, carrots, onions, cucumbers, and iris stems. When the organism was re-isolated from the rotted vegetables and inoculated into fresh slices, these were completely rotted. Decay took place from room

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temperatures up to 37° C. About the same time Madhok and Ud-Din (8) found a hitherto undescribed spore-forming bacterium capable of causing a fast-spreading rot in tomato fruits. This organism, which is Gram-positive and strictly aerobic, was named *Bacillus frutodestructuens*. More recently, Allen (1) has reported a strain of *Bacillus subtilis* to be capable of rotting potato cubes submerged in nutrient broth. In this latter case the conditions employed departed rather widely from those that potatoes are likely to encounter in the hands of the grower.

The main objectives of this study were to determine whether Alberta soils contain plant pathogenic strains of *B. polymyxa*, to ascertain the prevalence of such strains, and to determine under what conditions they may be destructive to potato tubers.

## Materials and Methods

### METHODS OF ISOLATION

The methods used in the isolation of *B. polymyxa* were, in general, similar to those employed by Ledingham *et al.* (7) and were based upon the ability of this organism to form spores and to ferment carbohydrates rapidly with the evolution of gas. In addition, the production of detectable amounts of acetylmethylcarbinol and considerable quantities of 2,3-butanediol was considered indicative of the presence of *B. polymyxa* when selecting this species from other bacteria capable of fermenting starchy material with the production of gas.

Soil, or other material to be tested for the presence of *B. polymyxa*, was treated as follows.

Samples of the material were suspended in sterile water and shaken for two or three minutes. Aliquots (1 ml.) of the sediment-free liquor were added, one per tube, to test-tubes of 14 mm. bore each containing 5 ml. of sterile water. These prepared tubes were then pasteurized by immersion in a boiling water-bath for 10 min. Samples (1 ml.) of the pasteurized material were used to inoculate 10 to 12 ml. lots of sterile wheat mash.

The wheat mash was prepared by placing 0.8 gm. of ground wheat in a test-tube and adding 10 to 12 ml. of water. The contents of the tube were then thoroughly mixed by shaking and were allowed to stand at room temperature for one and one-half to two hours. The tubes so prepared were then plugged and autoclaved for 30 min. at 15 lb. pressure.

After incubation at 35° C. for two to four days, mashes containing *B. polymyxa* show vigorous fermentation with the evolution of much gas, and when fermentation has proceeded for four to five days, negative iodine tests for starch are usually obtained.

When spent mashes showed positive tests for acetylmethylcarbinol using the method of O'Meara (9) and yields of 1.5 to 2% 2,3-butanediol, using the method developed by Corns (5), it was concluded that they contained *B. polymyxa*.

Dilution plates were prepared from the contents of the fermentation tubes using neutral red agar as employed by Ledingham *et al.* (7). Colonies of *B. polymyxa* absorb the dye from the agar and hence are easily distinguished from those of certain other bacteria that may be present. After following the routine just described the culture obtained at this stage is usually almost pure *B. polymyxa*.

The colonies selected from the above agar were streaked onto wheat-peptone-agar slants, which were found satisfactory for the propagation of *B. polymyxa*. The composition of this medium except for the wheat is as follows: agar—17 gm., peptone—3 gm., and water—1000 ml. These materials were heated in an autoclave for 5 to 10 min. at 15 lb. pressure. Tubes of the medium were then prepared by adding 0.5 gm. finely ground wheat to each, followed by 7 to 8 ml. of the liquefied peptone and agar mixture. Sterilization for 20 min. at 15 lb. steam pressure, cooling, and slanting of the medium were the final steps.

### Method of Determining Pathogenicity

Potato tubers were used in the pathogenicity tests. These were prepared and inoculated as follows.

Potato slices were prepared from whole, sound potato tubers that had been previously washed in water to remove all dirt and then surface-sterilized in a 1:1000 mercuric chloride solution. After removal from the solution the tubers were thoroughly rinsed in sterile water. Slices about one-quarter to one-half inch thick were removed from the tubers with a sterile knife and placed in sterile Petri plates containing a single layer of filter paper and 5 ml. water. Potato slices were inoculated by smearing the freshly cut surface with bacteria suspended in sterile water. The suspension was prepared by transferring a large loopful of the bacterial growth from a 24-hr.-old, wheat-peptone-agar slant culture of the organism to 5 ml. of sterile distilled water. When whole potatoes were employed, they were placed, following surface sterilization, in desiccators containing a few millilitres of water to prevent drying. The desiccators were then placed in thermo-regulated chambers at the desired temperatures for 24 hr. previous to inoculation to bring the potatoes to the cabinet temperature. Tubers were inoculated by stabbing with a flamed inoculating needle carrying bacteria obtained from a 24-hr.-old slant of the desired organism.

After inoculation, the tubers and slices were incubated for three to four days, after which notes were taken on their condition.

### Identification

The strains of *B. polymyxa* used in these studies fit the description given for this species by Bergey *et al.* (3) and by Porter, McCleskey, and Levine (10). The classification of Bergey *et al.* (3), which lists this organism as *Bacillus polymyxa* (Prazmowski) Migula, is followed here.

The identification is based on starch hydrolysis, formation of terminally located spores, negative Gram stain reaction, production of large quantities of 2,3-butanediol, and detectable amounts of acetylmethylcarbinol from carbohydrates. In addition the organism reduces nitrates to nitrites and produces acid, carbon dioxide, and hydrogen from a number of carbohydrates.

## Plant Pathogenic Strains of *B. polymyxa* in Alberta Soils

### OCCURRENCE AND PREVALENCE

Using the methods of isolation outlined above, the presence of *B. polymyxa* in Alberta soils was readily demonstrated. As indicated in Table I, it was obtained from all four of the main soil types of the province and from widely scattered points. Moreover, isolates made from a wide range of soil sources proved to be pathogenic to potatoes as was demonstrated by artificial inoculations, the results of which are recorded in the following and in subsequent sections.

TABLE I

STRAINS OF *Bacillus polymyxa* FOUND CAPABLE OF ROTTING POTATO SLICES AT 30° C. AND SOURCES OF THE ISOLATES

American Type Culture Collection isolates	N.R.C.* isolates	University of Alberta isolates			
		From leached grey wooded soils	From black soil	From dark brown prairie soil	From brown prairie soil
840	C25	U.A.175	U.A.228	U.A.152	U.A.179
7047	C42(2)	U.A.178	U.A.634	U.A.155	U.A.201
7070	C47		U.A.641	U.A.167	U.A.219
	C56(2)		U.A.647	U.A.183	U.A.220
			U.A.650	U.A.186	U.A.222
			U.A.646(2)	U.A.197	
				U.A.234	
				U.A.236	

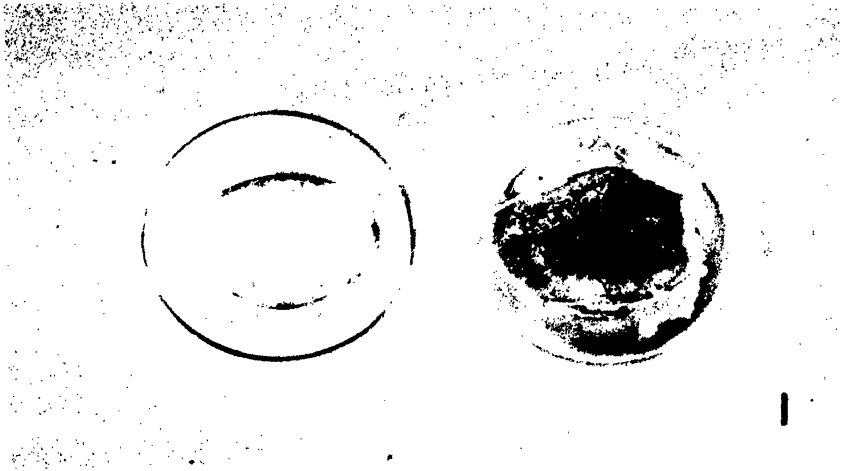
\* National Research Council of Canada.

NOTE: Sources of University of Alberta (U.A.) isolates: U.A. 152, Calgary; U.A. 155, Calgary; U.A. 167, Drumheller; U.A. 175, Dapp; U.A. 178, Perryvale; U.A. 183, Lethbridge; U.A. 186, Lethbridge; U.A. 197, Strathmore; U.A. 179, Taber; U.A. 201, Duchess; U.A. 219, Tilley; U.A. 220, Tilley; U.A. 222, Bow Island; U.A. 228, Olds; U.A. 234, Raymond; U.A. 236, Pincher Creek; U.A. 634, Edmonton; U.A. 641, Edmonton; U.A. 647, Edmonton; U.A. 650, decayed wood, Edmonton; U.A. 646(2), storm sewer discharge, Edmonton.

### EXPLANATION OF FIGURES

FIG. 1. Characteristic appearance of potato slices rotted by *Erwinia carotovora* (left) and *Bacillus polymyxa* (right).

FIG. 2. The effect of temperature upon the ability of three strains (left U.A.641; centre, C42(2); right, American Type Culture 7070) to rot whole potato tubers. Bottom row, 25°; second row, 30°; third row, 35°; top row, 45° C.





### RELATIVE PATHOGENICITY OF DIFFERENT SOIL ISOLATES

Potato tubers were chosen as host material for testing the relative pathogenicity of different strains of *B. polymyxa* for several reasons. Firstly, as noted above, Dowson (6) has reported the isolation of this organism from rotting potato tubers and its ability to rot potato slices. Secondly, potato tubers are produced in close contact with the soil, and thirdly they are grown all over the province in all four soil zones.

The strains studied included some 20 isolates obtained from soils from various parts of Alberta, and for comparison three strains from the American Type Culture Collection, namely 840, 7070, 7047, and a number of isolates made available from the collection of the National Research Council at Ottawa.

It can be seen from Table I that the ability to rot potato slices under the conditions of these experiments is possessed by all Alberta isolates of *B. polymyxa* tested, regardless of source, as well as by strains of this organism obtained from the American Type Culture Collection and the National Research Council of Canada. The different strains appeared to be approximately equal in virulence. Pathogenicity towards potato tuber tissue appears therefore to be quite generally characteristic of this organism.

### Comparative Effects of *B. polymyxa* and *E. carotovora* on Potato Tubers

Since *Erwinia carotovora* (Jones) Holland is the cause of a common bacterial soft rot of potatoes, a comparison of the effects of this organism with those produced by *B. polymyxa* should be of interest. These were studied under similar conditions.

Potato slices prepared as described under "Materials and Methods" were surface inoculated with active cultures of either *E. carotovora* or *B. polymyxa* and incubated at 30° C. for three days.

In the early stages of breakdown, *B. polymyxa* caused a jell-like rot filled with gas bubbles. The production of gas caused a slight increase in the volume of the slice during this stage of decay, which was followed by collapse (Fig. 1). The slices, depending upon the strain of *B. polymyxa* used, are reduced to a light or dark grey-coloured mass, which darkens upon ageing to almost black. A pleasant fruity odour arises from these slices.

*E. carotovora* reduced the potato tissue to the well-known creamy mass of a more or less sticky consistency. The breakdown product is cream-coloured and, when no other bacteria than *E. carotovora* are present, very little odour is produced.

### Effect of Temperature on the Rotting of Potato Tubers by *B. polymyxa*

Since temperature is one of the main factors to be considered in the storage of potatoes, it was decided to determine the temperature range over which potatoes are attacked by strains of *B. polymyxa*. The strains used included



representatives of those obtained from the American Type Culture Collection and the National Research Council of Canada, and two Alberta isolates.

Both whole and sliced raw potatoes were included in the temperature studies. These were prepared as previously described and were subjected to the temperatures indicated in Table II.

TABLE II

RELATIVE DEGREES OF ROTTING OF POTATOES BY *Bacillus polymyxa* AT DIFFERENT TEMPERATURES

<i>Bacillus polymyxa</i> strains used	Incubation temperature, °C.							
	15	20	25	30	35	40	45	50
Potato slices								
Check	—	—	—	—	—	—	—	—
7070	—	Tr.	+	+	+	+	+	+
C42(2)	—	—	+	+	+	+	+	+
U.A.641	—	Tr.	+	+	+	+	+	+
U.A.646(2)	—	—	+	+	+	+	+	+
Potato tubers								
Check	—	—	—	—	—	—	—	—
7070	—	—	—	+	+	+	+	+
C42(2)	—	—	—	—	+	+	+	+
U.A.641	—	—	—	—	+	+	+	+
U.A.646(2)	—	—	—	—	+	+	+	+

NOTE: Severity of rotting indicated as follows: — none; + slight to ++++ complete; tr. occasionally slight.

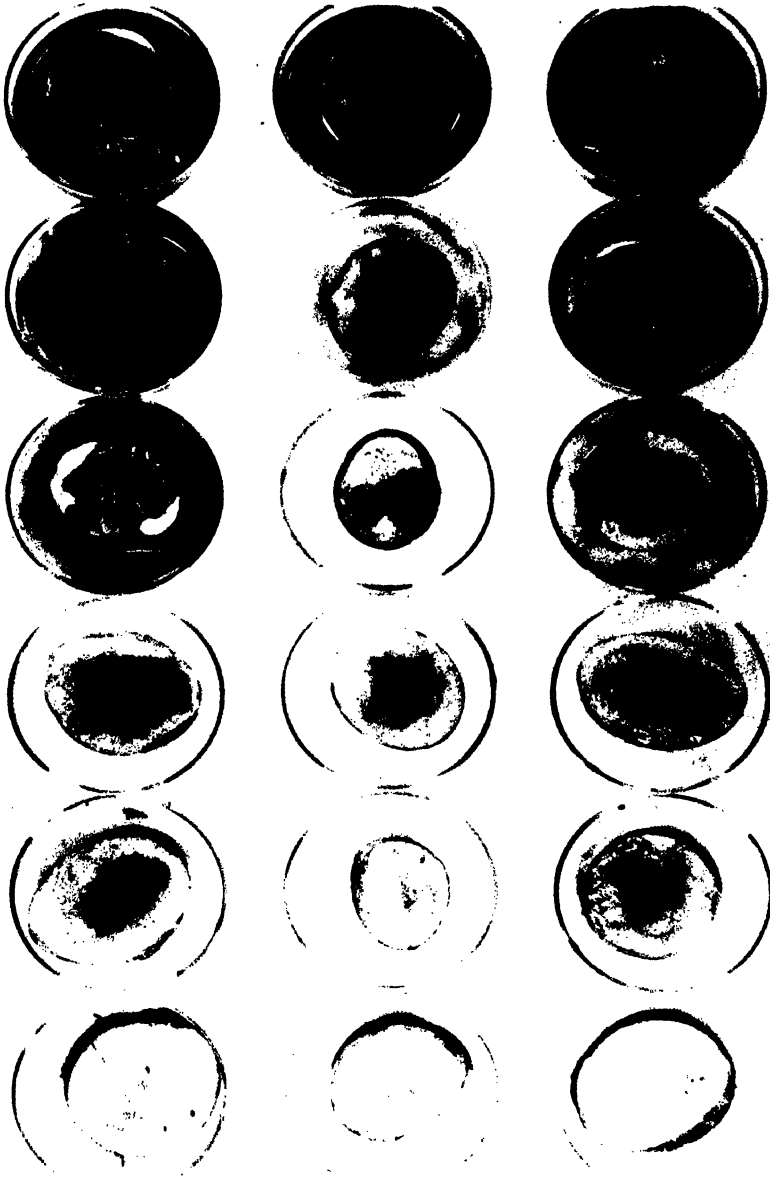
It is apparent from Table II that pure cultures of *B. polymyxa* are capable of rotting potato tuber tissue only at medium to high temperatures. Potato slices were rotted at temperatures from 20° to 45° C., whereas whole tubers were not rotted at temperatures below 30° C. The reason for this difference is not known (See Figs. 2 and 3).

Unpublished results obtained in this laboratory indicate that certain strains of *B. polymyxa* are capable of fermenting wheat mash at temperatures as low as 15° C. This indicates that *B. polymyxa* may be chemically active at temperatures considerably below 20° C. However, from the results shown in Table II, it appears that *B. polymyxa* is unable by itself to attack potato tissue at such temperatures. In fact, temperatures exceeding 30° C. would appear necessary before much damage to whole tubers could be produced by this organism. The maximum temperature at which the tuber slices and whole tubers were consistently rotted was 45° C.

#### EXPLANATION OF FIGURE

FIG. 3. Effect of temperature upon the ability of three strains (left, American Type Culture 7070; centre, C42(2); right, U.A. 641) to rot potato slices. Bottom row, 15°; second row, 20°; third row, 5°; fourth row, 30°; fifth row 35°; top row, 45° C.

PLATE II





### Isolation of *B. polymyxa* from Naturally Infected Potato Tubers

*B. polymyxa* has been isolated a number of times from rotting potato tubers that have been submitted to this laboratory. Although no instances of infection with this organism alone have been encountered in the material, it has, in most cases, been found in the actively rotting areas. The technique used for isolation was that described earlier in this paper under the heading "Methods of Isolation".

In addition, freshly cut portions of tubers, when buried in well moistened Edmonton soil in pots at temperatures of 20° to 25° C., became infected with *B. polymyxa* after four to six days' exposure. In one or two instances typical rot as caused by pure cultures of the organism was observed, but usually this bacterium occurred in mixtures with other bacteria and fungi.

### Discussion

Although it is demonstrated in this paper that strains of *B. polymyxa* capable of rotting potato tubers occur commonly in Alberta soils, this should not be the cause of undue alarm since it is also shown that this organism can rot potatoes only under rather restricted environmental conditions.

Temperature is shown to be a very important factor in influencing the ability of *B. polymyxa* to rot potato tissue. Fortunately this organism apparently is able to rot potato tubers only at relatively high temperatures. Potatoes may become contaminated with it in the field, but are not likely to be severely rotted by it there, at least under average soil conditions in Alberta. Although records (11) show that, to a depth of a half inch, the soil in this region sometimes reaches temperatures at which this organism can rot potatoes, the moisture supply will generally be too low at such times to permit damage.

It is possible, of course, since we have isolated this organism from mixed infections of tubers stored at temperatures below 20° C., that this species may be of more importance at low temperatures in mixed cultures with other organisms than alone.

It has recently been reported (12) that, where heating occurs in field-stored potatoes, temperatures as high as 44° C. are reached and that much of the damage done in such instances is due to bacterial action. *B. polymyxa*, in all probability, would be active.

Another factor to be considered, and one that is affected by temperature, is wound cork formation. Artschwager (2) has shown that suberization takes place at the surface of freshly cut tubers within 24 hr. at 21° C. It has been observed in the course of these studies that potato slices stored for a few hours after cutting are then not readily rotted by *B. polymyxa*. It is quite possible that the formation of wound cork may be, in considerable measure at least, the explanation for this.

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## INVESTIGATIONS ON RUBBER-BEARING PLANTS

### II. CARBOHYDRATES IN THE ROOTS OF *TARAXACUM KOK-SAGHYZ* ROD.<sup>1</sup>

BY PAUL R. GORHAM<sup>2</sup>

#### Abstract

The soluble carbohydrates were extracted, by means of hot water, from dried ground roots of *Taraxacum kok-saghyz* Rod. that had been extracted previously with acetone and benzene. A cleared portion of the extract served for the determination of hexose and, after invertase hydrolysis, sucrose. Another portion was subjected to mild acid hydrolysis before clearing, and served for the determination of total reducing value, whence fructosans, as inulin, were calculated. Separation of the free reducing sugars and sucrose from the fructosans by ethanol extraction proved unsatisfactory. Analyses of 171 one-year old roots from six crosses gave the following average values expressed as per cent dry weight: hexose 1.6, sucrose 4.7, and inulin 41. The analytical data suggest the possibility of selecting and breeding for strains of kok-saghyz capable of high carbohydrate production.

#### Introduction

The physiological and biochemical aspects of carbohydrate production and change in *Taraxacum kok-saghyz* Rod. have aroused considerable interest in connection with agricultural practices used with this plant. The carbohydrates have been of industrial concern as well, since their accumulation in the root is closely associated with that of rubber, and the carbohydrate content is sufficiently high to suggest alcohol production as a valuable adjunct to the process of rubber extraction. A recent review (13) covers much of the literature dealing with carbohydrates in kok-saghyz.

The carbohydrates of one-year old plants are mainly represented by inulin and reportedly comprise 12.6% on a fresh weight basis or 42.0% on a dry weight basis (4, 12). One ton of fresh roots yields about 60 litres of alcohol (4). It is estimated that for every ton of crude rubber extracted from kok-saghyz, two tons of alcohol (expressed as absolute alcohol) can be obtained from the inulin extracted by diffusion batteries of the type used for sugar beet (12).

The carbohydrate changes in kok-saghyz associated with development, maturation, and periodic fertilizer applications have been investigated (2, pp. 56-70; 8-11; 14, pp. 89-98; 18; 19; 20-22; 23, pp. 5-29). During the period of intensive growth, leaf carbohydrate content is low. With biological maturation, the rate of photosynthesis slows down and soluble carbohydrates accumulate in the leaves; inulin in the roots. This is accompanied by increased formation and storage of rubber. Supplying nitrogen at maturity stimulates further growth of the leaves, and consequently decreases the accumulation of

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both rubber and carbohydrates in the roots. However, rubber formation and accumulation bears no direct relation to photosynthesis (25-27).

After freshly harvested roots have been stored for two months, the inulin almost completely disappears and a considerable accumulation of mono- and disaccharides takes place (3). The exhaustion of carbohydrate reserves by developing leaves of kok-saghyz inhibits root initiation (24). The degree of survival of cuttings from roots in storage is correlated with their carbohydrate content (17).

Physiological studies concerned with the propagation of kok-saghyz by means of root cuttings made it desirable to have a procedure for carbohydrate analysis that would be suitable for routine application.

### Materials and Procedure

Roots of individual seedlings from six crosses (four reciprocal), grown in an outdoor plot at Ottawa for five months prior to harvest\*, were used for the study of carbohydrate content. These were dried at 40° C. and stored until analyses were undertaken. Each root was ground in a Wiley cutting mill, Intermediate Model, to pass a 20-mesh screen. The samples ranged in size from 1 to 5 gm. A few samples exceeding 5 gm. in weight were analysed in parts and the results totalled. All samples were extracted successively with acetone and benzene for resin and rubber hydrocarbons and the residual solvent allowed to evaporate before extraction of carbohydrates was undertaken.

Initially, attempts were made to separate the carbohydrates according to the method of Loomis and Shull (16, p. 281). This involved Soxhlet extraction for six hours with 85% ethanol for the removal of free reducing sugars and sucrose, followed by five successive hot water extractions for the removal of fructosans. This method was found to have several disadvantages, but these were overcome by adopting a procedure employing only hot water extraction.

Each sample, wrapped in a fluted filter paper as removed from the Soxhlet thimble, was placed in a 250 ml. beaker and 50 ml. of boiling water poured over it. This was placed on a multi-heat hotplate and maintained, with constant stirring, at a temperature of 90° to 95° C. for 30 min. The hot extract was carefully decanted, with as little transfer of sample as possible, into a funnel fitted with a 15 cm. fast-filtering paper. The filtrate was collected in a 250 ml. volumetric flask. Approximately 40 ml. of boiling water was added to the residue in the beaker and heated for another 15 min. This extract was then decanted into the same filter. The process was repeated three times more, after which experimental determination showed that little, if any, carbohydrate remained. With the last extraction, the sample was transferred to the filter and washed with a small amount of boiling water.

\* Crosses were made and seedlings started in the greenhouse during the winter months of 1942-43. This work, as well as the subsequent analyses of the roots for resin and rubber hydrocarbons, was conducted by Dr. W. H. Minshall.

The combined filtrates (I) were cooled, adjusted to volume, and aliquots withdrawn for analyses. Root samples of less than 1 gm. in weight were extracted in a total of 100 ml. instead of 250 ml. Routine extractions and analyses were conveniently carried out on lots of six samples.

Fifty millilitres of I were cleared and decolorized according to the method of Hassid (6). The amounts of saturated neutral lead acetate and disodium phosphate recommended by Hassid for clearing ethanol-soluble extracts were found to cause low reducing values when applied to aqueous extracts. Possibly an excess of phosphate interfered with the stoichiometric reduction of ferricyanide (1). Satisfactory reducing values, as determined by recovery experiments, were obtained by the use of 1 ml. of saturated neutral lead acetate followed by 2 ml. of saturated disodium phosphate. The cleared and decolorized extract (II) was made up to 100 ml., and served for determinations of sucrose and free reducing sugars.

Twenty-five millilitres of I were pipetted into a 50 ml. Erlenmeyer flask, 0.25 ml. of concentrated hydrochloric acid added, and the flask suspended for 35 min. in a water-bath at  $70 \pm 0.2^\circ \text{C}$ . The acidified extract was cooled to room temperature, neutralized to litmus with 3*N* sodium hydroxide\*, cleared and decolorized as above, and made up to 100 ml. This acid-hydrolysed extract (III) served for the determination of inulin.

The hydrolysis of the fructosans was carried out prior to clearing in order to avoid the possibility of inulin being carried down with the lead precipitate. Conditions of mild acid and moderate temperature were employed in order to minimize the hydrolysis of pectin, gums, or glucosans that were present. These conditions were found adequate, however, for the complete hydrolysis of sucrose, thereby providing a means for the determination of the total reducing value.

The ferricyanide-ceric-sulphate method of Hassid (7) was employed for the determination of reducing sugars. Replicate analyses carried out initially agreed so closely that for routine purposes single determinations for each carbohydrate were deemed adequate. Aliquot sizes were reduced and runs repeated whenever heating produced complete decolorization of the ferricyanide.

#### *Hexose*

The amount of free reducing sugars, expressed as hexose, was determined on a 5 ml. aliquot of II.

#### *Sucrose*

Hydrolysis of 25 ml. of II was carried out in a 50 ml. volumetric flask. The pH was adjusted to 4.5 with 10% acetic acid, using methyl red as internal indicator, and two drops of a 1% aqueous suspension of invertase were then

\* About 0.75 ml. were required. In most cases, the end-point of the titration was determined by the appearance of the pronounced yellow colour of a naturally occurring indicator. Litmus was used only when some obscuring pigment made this end-point uncertain. Excess alkali was found to interfere with clearing and to cause greatly reduced analytical values.



added. After 22 hr. at room temperature, sufficient for complete hydrolysis, the volume was made up to 50 ml. and the combined reducing value of the hydrolysed sucrose plus free reducing sugars was determined on an aliquot of 5 ml. A correction was applied for the slight reducing value of the invertase. The sucrose content was determined by multiplying the difference between the free reducing value and the combined reducing value per sample by 0.95, the ratio of one gram-mole of sucrose to two gram-moles of hexose.

### *Inulin*

The total reducing value was determined on a 1 ml. aliquot of III. The fructosan content, expressed as inulin (assuming the formula  $(C_6H_{10}O_5)_6 \cdot H_2O$ ), was determined by multiplying the difference between the combined reducing value and the total reducing value per sample by 0.916.

## Results and Discussion

In the initial trial of the method of Loomis and Shull for separating the carbohydrates the first disadvantage was that the aqueous extract following ethanol extraction was found to contain hexose to the extent of 9 to 19% of the total found in both extracts (Table I). This would have necessitated a hexose determination on each extract.

TABLE I

TOTAL HEXOSE CONTENT OF DRIED KOK-SAGHYZ ROOTS DETERMINED BY ETHANOL EXTRACTION FOLLOWED BY WATER EXTRACTION

Sample	Dry wt., gm.	By ethanol, %	By water, %	Total, %	Per cent of total in water extract
S64.1	4.406	1.20	0.29	1.49.	19
.2	3.235	1.35	0.13	1.48	9
.3(a)	4.356	1.14	0.21	1.36	15
(b)	3.452	1.29	0.28	1.57	18
.4	1.809	2.48	0.31	2.79	11

The second disadvantage was that part of the fructosans were extracted by the 85% ethanol and erroneously determined as sucrose. Evidence of this was provided by the observation that sucrose values obtained after invertase hydrolysis of ethanol-soluble extracts were appreciably lower than those obtained after acid hydrolysis. The observed differences ranged from 1.5 to 4.3% (Table II), and were not caused by incomplete hydrolysis with invertase since values determined after 40 hr. differed little from those determined after 24 hr.

A third disadvantage, from the standpoint of routine analysis, was the additional time required for the two types of extraction.

The maximum, minimum, and mean values, in per cent, for the hexose, sucrose, inulin, and total soluble carbohydrates found in the seedling roots

from six crosses are summarized in Table III. The hexose content was consistently low in all crosses, with mean values ranging from 1.3 to 2.2%. Attempts to evaluate the glucose and fructose content by means of a modified

TABLE II

SUCROSE DETERMINATIONS UPON THE ETHANOL-SOLUBLE EXTRACT OF DRIED KOK-SAGHYZ ROOTS AFTER HYDROLYSIS WITH HYDROCHLORIC ACID OR WITH INVERTASE

Sample	Dry wt., gm.	Sucrose, %			Difference, %
		Hydrochloric acid	Invertase		
			24 hr.	40 hr.	
S66.19	3.564	13.0	9.7	9.6	3.3
.20	1.851	13.1	8.8	8.6	4.3
.21	2.892	11.9	9.2	9.1	2.7
.22	2.093	8.9	7.4	7.0	1.5
.23	3.074	6.8	4.7	4.8	2.1
.24	0.849	13.1	8.8	8.5	4.3

TABLE III

MEAN VALUES, TOGETHER WITH THE MINIMUM AND MAXIMUM VALUES OBSERVED, FOR THE HEXOSE, SUCROSE, INULIN, AND TOTAL SOLUBLE CARBOHYDRATES CONTENT OF SEEDLING ROOTS FROM SIX CROSSES OF *Taraxacum kok-saghyz*

Cross	No. roots analysed	Dry wt., gm.	Hexose, %	Sucrose, %	Inulin, %	Total sol. carbo- hydrates, %
253.2 × 181.8	24	2.243	1.9	4.7	41.3	47.9
		0.659-6.263	0.8-3.0	2.1-8.7	21.6-51.3	28.1-57.3
253.2 × 253.3	6	2.068	1.5	6.3	46.9	54.7
		1.279-3.156	1.2-1.8	5.7-6.8	46.0-48.1	53.4-56.3
253.2 × 257.1	32	1.121	1.4	5.3	43.0	49.7
		0.083-4.398	0.6-2.4	1.8-10.5	23.8-50.7	27.1-57.2
	21	3.087	1.9	5.4	40.9	48.2
		0.659-6.195	1.3-3.0	3.2-8.9	38.1-48.1	44.9-57.0
257.1 × 93.24	14	1.074	1.3	3.9	41.8	47.0
		0.073-3.072	0.5-2.4	1.4-6.1	33.4-53.2	39.2-59.6
	17	1.746	1.5	3.3	41.7	46.5
93.24 × 150.11	5	0.075-3.057	0.5-2.4	0.3-4.7	29.1-59.5	35.5-62.2
		1.118	1.5	2.0	33.9	37.4
	13	0.318-1.894	1.3-1.8	0.0-3.9	24.2-37.8	27.0-42.5
		2.110	1.7	6.5	32.7	40.9
150.11 × 253.2	5	0.843-3.025	1.0-2.1	4.3-8.3	23.1-40.4	33.1-48.5
		1.397	2.2	-	40.2*	42.5
	34	0.408-3.586	1.4-3.6	-	30.4-47.7*	32.0-49.1
		2.388	1.5	-	42.7*	44.2
		0.198-6.154	0.8-2.7		13.3-63.9*	15.0-64.6

\* Combined sucrose and inulin.

ferricyanide procedure resembling that of Becker and Englis (1, 5) were not wholly satisfactory. However, the results indicated the presence of both glucose and fructose in either approximately equal proportions or with fructose predominating. Throughout the various crosses the mean contents ranged from 2.0 to 6.5% for sucrose, 32.7 to 46.9% for inulin, and 37.4 to 54.7% for the total soluble carbohydrates.

The mean values found for the three types of carbohydrate compare favourably with those reported by others (8, 18, 22). The sucrose and inulin content exceeds that found in the roots of our native dandelion, *Taraxacum officinale* Weber (15). This species contains reducing sugar 1.2%, sucrose 3.5%, and polysaccharides, consisting of approximately equal parts of fructosan and dextrin, 10.7%, calculated on a dry-weight basis. There is little or no inulin present. In kok-saghyz, the fraction reported as inulin undoubtedly contained a considerable proportion of lower molecular weight fructosans. This is suggested by the ethanol-soluble, acid-hydrolysable carbohydrate that was unaffected by invertase (Table II).

Differences in the growth of the seedlings, as evidenced by variation in root dry weight, were considerable (Table III). However, carbohydrate content was not correlated with root weight. Although the mean values for the total soluble carbohydrates in reciprocal crosses agreed quite well, comparisons as to carbohydrate production by specific crosses are of little significance because of the great variation in content and numbers of plants surviving within each cross. However, the data suggest that selecting and breeding for strains capable of producing large amounts of carbohydrate is feasible.

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## INVESTIGATIONS ON RUBBER-BEARING PLANTS

### III. DEVELOPMENT OF NORMAL AND ABORTING SEEDS IN *ASCLEPIAS SYRIACA* L.<sup>1</sup>

By RAYMOND J. MOORE<sup>2</sup>

#### Abstract

A low percentage (2 to 3%) of the flowers of *Asclepias syriaca* L. produce mature seed pods. Many ovaries begin to enlarge but abort before reaching maturity. Histological examination of these pods shows that ovule abnormalities accompany pod collapse. Ovules in free-nucleate endosperm stages show a growth of the integument that is excessive for ovules at this stage of embryo sac development. Older aborting ovules contained a zone of collapsed cells completely enclosing the endosperm.

Seed collapse of the former type at least appears similar to cases attributed to a competition for food between endosperm and the adjacent maternal tissue of the ovule, termed "somatoplastic sterility" by Brink and Cooper.

#### Introduction

Early in 1942 the Division of Botany and Plant Pathology of the Department of Agriculture, in co-operation with the National Research Council of Canada, initiated a comprehensive series of investigations on plants that were potential supplementary sources of natural rubber. This Division was responsible for study of the cytogenetics, physiology, and agronomy of these plants as a means of determining whether they could be economically grown in Canada.

One of the species that received major attention was *Asclepias syriaca* L., common milkweed. Wartime interest in this species also developed as a result of the use of milkweed floss as a substitute for kapok in life-preserving equipment. Early observation showed that very few milkweed flowers produced mature pods. This fact was of great importance from the standpoint of utilization of floss and also from that of rubber production since large quantities of seed were needed to plant commercial acreages. The investigation reported below was undertaken as an attempt to explain the failure of many flowers to produce pods.

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### Field Observations

Observations of the growth of seed pods revealed that only 2 to 3% of the flowers produced mature fruits. A high percentage of the flowers fell 10 to 12 days after flowering. These flowers showed no enlargement of ovaries or of flower pedicel. Ovaries that survived this period began growth, but all did not reach maturity. Death of these ovaries occurred at widely varying stages. Ovaries showing only slight enlargement as well as pods as long as 6 cm. dried and shrivelled up. These however did not fall from the plant but remained firmly attached to the peduncle by the thickened pedicel. The percentage of enlarged ovaries that failed varied widely; all or none or any intermediate fraction of the developing pods in one umbel might abort at any stage of growth and at periods up to two and one-half to three weeks after flowering.

### Materials and Methods

Material for histological study was obtained from plants growing in experimental plots in the Dominion Arboretum and Botanic Garden, Ottawa, Canada. The plants were grown from seed collected at several stations in and near Ottawa. The soils of the various plots used were clay-loam and loam in nature.

All plantings flowered freely during July of their second year and fixations were made during July and August of 1944 and 1945.

Ovules of normally developing pods and of enlarged but apparently aborting ovaries were fixed in Randolph's Nawashin, embedded, and prepared for microscopic study in the usual manner. Safranin and fast green, with the occasional addition of gentian violet, and Heidenhain's iron alum haematoxylin were the stains used.

### Literature Review

Frye (7) has described the development of the ovule and megagametophyte and followed the development of the fertilized embryo sac as far as the initiation of the cellular condition of the endosperm. A single integument and a deeply sunken nucellus constitute the maternal tissues of the ovule. A hypodermal cell of the nucellus functions directly as the megaspore mother cell and from this cell a row of four megaspores is formed by the meiotic divisions. Usually, but not always, it is the innermost of these that develops into an eight-nucleate, seven-celled embryo sac of the Normal-Type (11). The mature embryo sac therefore contains a flask-shaped egg cell, two linear synergids, a large vacuolate two-nucleate primary endosperm cell, and three antipodal cells. The polar nuclei may or may not fuse before fertilization occurs. In the present study fusion before fertilization was more commonly seen. The nucellus is early absorbed by the embryo sac and none is present at maturity.

Double fertilization occurs and is immediately followed by division of the endosperm nucleus. Endosperm development is of the free-nucleate type, the early mitoses producing free nuclei that lie in a peripheral layer of cyto-

plasm about the central vacuole of the endosperm cell. At the fourth series of mitoses, by which 16 nuclei are formed, wall formation begins and thereafter the endosperm is multicellular. At this time, the zygote, until now undivided, divides by transverse wall.

### Development of the Normal Ovule

The present study agrees with that of Frye as to the structure of the mature ovule and early postfertilization development. Up to the eight-nucleate endosperm stage there has not been a great increase in the size of either the ovule or embryo sac, these being  $100\mu \times 150\mu$  and  $30\mu \times 100\mu$  respectively. Nor has increase in the thickness of the integument become conspicuous, for during the free-nucleate stage of the endosperm development this remains about six cells thick lateral to the embryo sac (Fig. 3). A strand of elongate cells along the funicular side of the ovule passes to the level of the antipodal end of the embryo sac. No differentiated vascular tissue is present in this strand and it is not connected directly with the embryo sac.

Rapid growth of the integument occurs after the endosperm has become septate and the zygote begins to divide. Early proembryos (Fig. 9) were found in pods 15 to 20 mm. in length. The integument (Fig. 8) was by now quite thick and the tuft of floss well begun.

In Fig. 14, a 4.5 mm. seed of a 40 mm. pod, it will be noted that the endosperm is a tissue of considerable size, at the micropylar end of which lies a spherical embryo (Fig. 13) with a multicellular suspensor. The endosperm cells are generally large and vacuolate with no reserve food. The integument shows some differentiation into layers. A true vascular strand, five to six cells thick, extends beneath the epidermis along the funicular side to the level of the chalazal end of the endosperm and there ends with no definitely differentiated zone connecting it with the embryo sac. Within the vascular strand or in regions where no vascular tissue is present immediately beneath the epidermis is a region about seven cells thick of compact, smaller, roughly isodiametric parenchyma. The cells lying within this compact zone are larger and more loosely packed and constitute a region about 7 to 14 cells thick. Adjacent to the endosperm is an area of two to three layers of empty, collapsed cells. Continuous with the micropylar end of the embryo sac and extending through the mid region of the integument to the region from which the floss arises is a channel of elongate usually empty cells.

Seed of 110 mm. pod presents a similar, though more advanced picture. At this time the embryo is greatly enlarged and shows a well formed radicle and cotyledons. It is embedded in endosperm the cells of which are thin-walled and contain dense protoplasts, apparently well supplied with reserve food material. This is essentially the condition found in the mature seed. The integument is differentiated into the same regions as previously described, epidermis, vascular strand, compact and loose parenchyma (less definitely distinguishable here), and an inner zone of collapsed cells extending in a central channel to the floss region.



The seed coat of a mature seed consists of two distinct layers.

1. Peripheral corky layer, one cell thick.
2. Thicker multicellular layer of remnants of walls of collapsed cells. In this layer traces of the vascular strand can be recognized along the funicular side.

The corky layer originates from the epidermis of the integument and the thicker layer from the collapsed cells of the integument from which the protoplasts have been absorbed by the endosperm.

### Floss Development

A tuft of silky hairs (the milkweed floss of commerce) is found on one end of the flat oval-shaped mature seed.

The origin of these hairs has been described by Frye (7) and by Netolitzky (9). They arise at the placental end of the ovule between the base of the funiculus and the micropyle when the ovule, as seen in longitudinal section, has become elongate and roughly rectangular. Each hair results from the elongation of an epidermal cell of the integument (Fig. 15). The epidermal cells become richly protoplasmic and elongate rapidly. The nucleus moves out to the mid region of the cell but no division follows and the mature hair is a uninucleate single cell.

Floss development is initiated slightly earlier, relative to the embryo sac stage, in abortive than in normal ovules. In the former, cell elongation begins at the eight-nucleate endosperm stage (Fig. 4) and, in normal ovules, when the ovule has become approximately the size of the abortive ovule containing free-nucleate endosperm. Normal ovules of this size contain cellular endosperm and early proembryos.

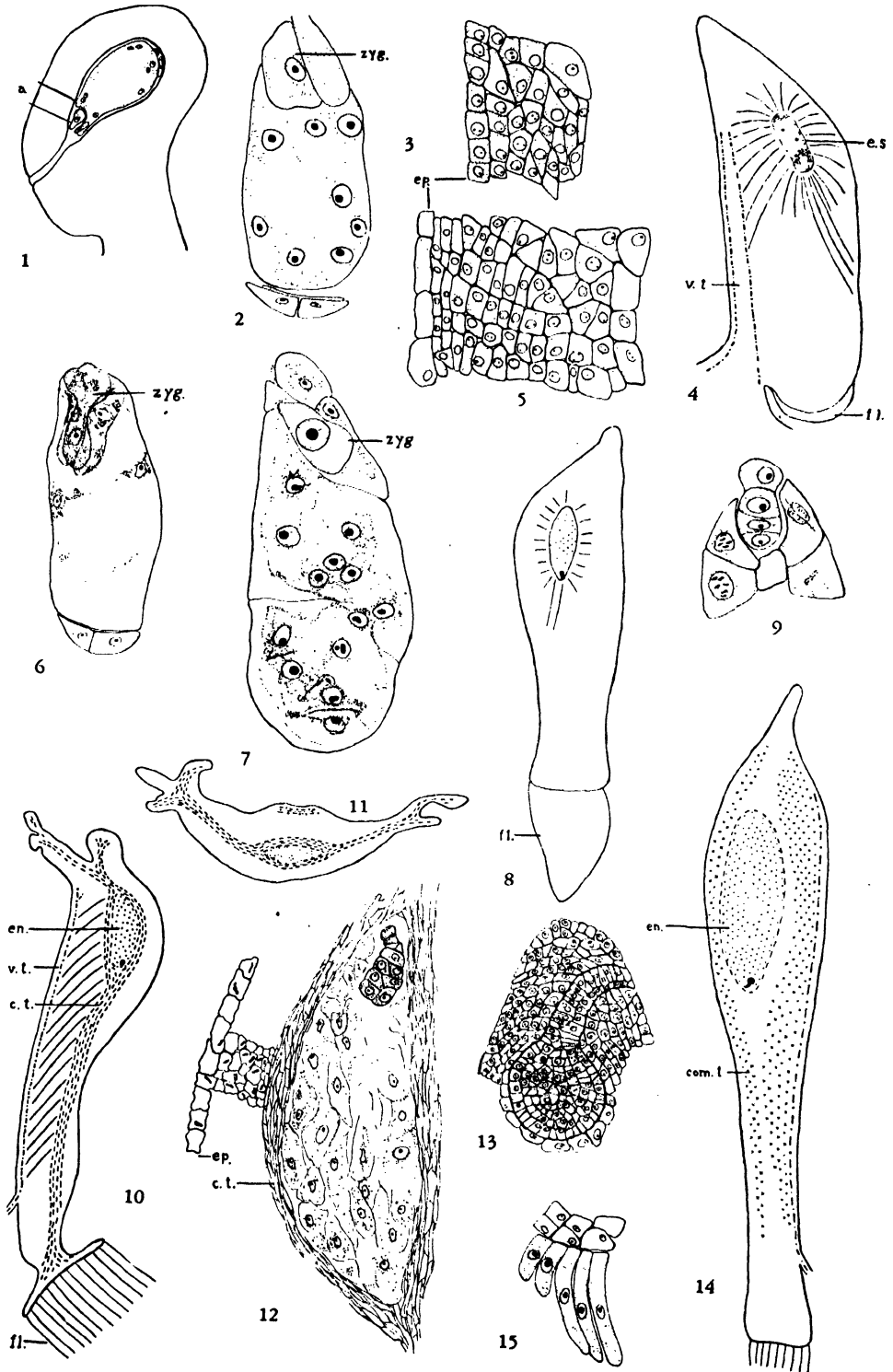
### Development of Ovules in Aborting Pods

Pods that were slow growing or appeared shrunken or yellowish were fixed for histological study. These ranged in size from those scarcely larger than the mature ovary to pods 2 cm. in length.

#### EXPLANATION OF FIGURES

FIG. 1. Normal ovule, eight-nucleate endosperm stage.  $\times 185$ . FIG. 2. Normal embryo sac, eight-free-nucleate endosperm.  $\times 450$ . FIG. 3. Section of integument of Fig. 1, in region "a".  $\times 490$ . FIG. 4. Hyperplastic ovule, eight-nucleate endosperm stage.  $\times 90$ . FIG. 5. Section of integument of Fig. 4, corresponding to Fig. 3.  $\times 490$ . FIG. 6. Abnormal embryo sac at two-nucleate endosperm stage, from hyperplastic ovule.  $\times 450$ . FIG. 7. Abnormal partially septate embryo sac at 16-nucleate stage, from hyperplastic ovule.  $\times 450$ . FIG. 8. Normal ovule at four-celled proembryo stage.  $\times 50$ . FIG. 9. Normal four-celled proembryo, with adjoining endosperm.  $\times 410$ . FIG. 10. Aborting ovule with multicellular endosperm and embryo and zone of collapsed cells.  $\times 50$ . FIG. 11. Transverse section of ovule in Fig. 10 at level of endosperm.  $\times 50$ . FIG. 12. Endosperm, embryo, collapsed tissue, and portion of integument of ovule in Fig. 10.  $\times 200$ . FIG. 13. Embryo and adjoining endosperm of ovule in Fig. 14.  $\times 200$ . FIG. 14. Normal ovule from 40 mm. pod.  $\times 20$ . FIG. 15. Epidermal cells of integument elongating to form floss.  $\times 325$ .

LEGEND: com.t.—compact tissue; c.t.—collapsed tissue; en.—endosperm; ep.—epidermis of integument; e.s.—embryo sac; fl.—floss; v.t.—vascular tissue; zyg.—zygote.



The ovules of these pods contained embryo sacs in both the free-nucleate and cellular endosperm stage. All ovules with free-nucleate endosperm and undivided zygote were distinguished from normally developing ovules at the corresponding stage of embryo sac development by the excessive growth of the integument that had occurred prematurely (Fig. 16).

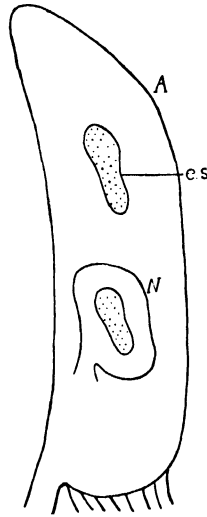


FIG. 16. Outlines of normal ovule (N) and of aborting ovule (A), both at eight-nucleate endosperm stage. Both ovules drawn to same scale to show relative sizes.

The integument was composed of thin-walled undifferentiated cells. In the zone about the embryo sac the cells, roughly rectangular in section with the longer axis parallel to the margin of the embryo sac, were arranged in regular compact rows appearing as if radiating from the periphery of the endosperm. Distal to this compact zone the cells were larger, roughly isodiametric, and not oriented in any special manner. Generally the cytoplasm of the cells of the former region stained more densely and the nuclei were larger than those of the looser parenchymatous region. The embryo sacs of most such ovules appeared abnormal and obviously degenerate.

Aborting ovules containing multicellular endosperm and embryos were characterized by a zone of empty collapsed integument cells that enclosed the embryo sac on all sides (Figs. 10, 11) and extended through the middle of the integument into the wings. The embryo sacs were in an obvious state of breakdown, the endosperm cells being shrunken and separated from one another. The embryo was as yet in an apparently more normal condition than the endosperm.

Ovules of a single pod were at similar stages of integument and embryo sac development. Hyperplastic ovules and unfertilized ovules occasionally were found in the same pod but as yet no such occurrence of hyperplastic and normal ovules has been seen.

Abnormality of the free-nucleate endosperm was apparent in the distorted shape of the nuclei and in the shreddy and coagulated appearance of the cytoplasm (Fig. 6). No doubt this appearance was exaggerated by fixation but probably an unusual physiologic condition of the protoplast was the basis of the abnormal reaction to the fixing fluid. Embryo sacs of normal ovules were not plasmolysed in this manner. Occasionally a partially-septate condition of the endosperm cell at the 8- to 16-nucleate stage was seen (Fig. 7).

### Description of Specific Cases of Aborting Ovules

Abortion may set in immediately after fertilization. Such was the case of an ovary 2.5 mm. in length. The ovules,  $150\mu \times 200\mu$  in size, contained embryo sacs with undivided zygotes and endosperm in the one- to two- or four-free-nucleate stage. The integument was 11 to 12 cell layers thick lateral to the sac, about twice the thickness of that of corresponding normal ovules. The endosperm was obviously in a state of disintegration, the cytoplasm being badly distorted by fixation and shrunk into coarse strands and blotchy masses. Some of the nuclei were faint in outline, as though the nuclear membrane had broken down. Frequently the zygote was disintegrating and appeared as a dense shrunk mass of cytoplasm in the midst of which a darker spot, probably the remnant of the nucleus could be seen. The synergid cells were represented by elongate masses of darkly staining cytoplasm. An embryo sac with two-nucleate endosperm is shown in Fig. 6. As in comparable normal ovules, no breakdown of the antipodal cells had yet occurred in such ovules. Occasionally a small deposit of apparently extracellular debris was seen between the antipodal cells and the adjoining integument cells. The debris did not appear to be the remnant of collapsed cells.

Aborting pods containing ovules in later stages of development also were characterized by the conspicuous premature thickening of the integument and an abnormal condition of the endosperm. The 4- to 16-nucleate endosperm stage was found in ovules the dimensions of which fell within the range of  $150$  to  $200\mu \times 400$  to  $650\mu$ . The embryo sacs measured  $30$  to  $50\mu \times 60$  to  $100\mu$ . The integument was 11 to 19 cell layers thick lateral to the embryo sac and 20 to 28 cells deep at the antipodal end. No true vascular tissue was present in ovules aborting in these early stages but a strand four to six cells thick of undifferentiated, elongate cells extended along the funicular side to the level of the antipodal end of the embryo sac. Conspicuous deposits of reserve food in the integument were lacking. With respect to the vascular development, collapsing seeds were similar to early normal ovules.

The extent to which disintegration of the endosperm had progressed varied somewhat in different ovules. Often the endosperm was not visibly abnormal but from the condition of the integument it could be safely judged that the ovules were destined to die before maturity. Disorganization of the endosperm was to be noticed in the distorted and plasmolysed condition of the tissue similar to that described for earlier stages.

Occasionally a partially septate condition of the endosperm at the 16-nucleate stage was noted (Fig. 7). In normal ovules the endosperm becomes septate at this time whereas in aborting ovules the 16-nucleate endosperm cell was divided into three to four irregular cells. The nuclei were embedded in coarse blotchy masses of cytoplasm and between some pairs there was an appearance of a spindle arrested in the process of cell-plate formation. The cells of the integument bordering on these ovules were not collapsed. Up to the 16-nucleate endosperm stage the zygote was undivided. No food reserve was noticeable in the endosperm.

Larger collapsing pods contained ovules with multicellular endosperm and early embryos. As previously mentioned, these ovules were characterized by a conspicuous zone of empty collapsed cells enveloping the embryo sac, as seen in Fig. 10 in longitudinal section and in Fig. 11 in transverse section. This condition was seen in ovules of failing pods 0.3 to 2 cm. in length.

Collapsed cells constituted the layers of the integument immediately adjacent to the embryo sac. Distal to this zone lay the yet normal cells of the integument and epidermis. The width of the collapsed zone and of the region of normal parenchymatous integument varied in different ovules, according to the extent to which seed collapse had progressed. In the extreme, no normal integument remained, all the cells having gone into the formation of the dead zone enclosing the embryo sac. The protoplasts of this central zone of integument are normally less dense than those of more peripheral cells in advanced ovules but the existence of such a zone of collapsed tissue at such an early stage of embryo sac development is definitely abnormal. A well-developed vascular strand, containing differentiated vascular cells passed down the funicular side of the ovule to the level of the antipodal end of the embryo sac. The roughly isodiametric cells of the integument were oriented in well-defined rows, passing obliquely from the vascular bundle to the periphery of the collapsed zone.

As seen in Fig. 12 the endosperm was in an advanced state of breakdown, the individual cells being shrunken and distorted and separated from one another. The multicellular proembryo, which consists of a few-celled suspensor and globular embryo proper, appeared to be in a relatively healthier condition than the endosperm. To judge by appearance, the endosperm failure seemed to precede that of the embryo, a condition frequently noted in studies on collapsing seeds.

### Unfertilized Ovules

Unfertilized ovules are readily distinguishable from aborting seeds. In the former no evidence of activity is visible. The egg cell and primary endosperm nucleus remain undivided and no development of the integument either by cell multiplication or cell enlargement takes place. Breakdown of the ovule finally follows, being first noticeable in the starved appearance of the integument cells. As degeneration proceeds, the integument cells, first those in the regions remote from the funiculus, empty and the walls shrink and collapse. Collapse of the embryo sac also shortly occurs.

## Discussion

It has frequently been observed in orchard fruits that a considerable percentage of flowers fail to produce mature fruits. This unsuccessful group is made up of ovaries that apparently never began development, and, as well, many ovaries that had enlarged but died before reaching maturity.

Studies of fruit development of orchard fruits have shown that the drop of immature fruits occurs in a number of distinct waves. Examination of the fruits has revealed that abnormality and early degeneration of the macrogametophyte and lack of fertilization are responsible for part of the drop. However, in a high percentage, definite evidence of fertilization and early embryo growth was reported.

A considerable number of studies on fruit collapse in pear, apple, plum, sour cherry, sweet cherry, and grape have been reviewed by Brink and Cooper (3, 5). Visible evidence of the cause of the seed failure was not found in some cases, whereas in others an excessive development of one of the maternal tissues of the ovule was observed.

In extensive investigations of seed abortion in alfalfa, Brink and Cooper (1, 2, 4, 6) found that such an excessive growth of the maternal tissue adjacent to the embryo sac occurred in failing ovules. These ovules showed unusual growth of the integuments and, apparently following this, collapse of the endosperm. Last of all, the embryo breaks down.

Such hyperplasia of the maternal tissue adjacent to the macrogametophyte has been termed "somatoplastic sterility" by Brink and Cooper (1). Comparable phenomena were found to accompany seed collapse after certain interspecific crosses of *Nicotiana*, *Petunia*, and *Lycopersicon* (5), of *Datura* (10), and of *Nicotiana* (8).

Brink and Cooper considered that a competition for the available food supply takes place between the endosperm and the adjacent ovular tissue. The ability to utilize the nourishment is proportional to the rate of nuclear division. In ovules that aborted, the rate of endosperm nuclear division was observed to be slower than that of ovules that reached maturity. The slower growth rate left more food available to the maternal tissue, which was thus stimulated to excessive development.

As a result of the competition for food the physiologically non-vigorous endosperm was starved and death of the embryo followed. Since the breakdown of the embryo appeared always to follow that of the endosperm, the endosperm failure was held to be the primary cause of seed collapse.

The lack of vigour of the endosperm was attributed to its unfavourable genetic constitution, received in the combination of incompatible genomes following interspecific pollinations or to a homozygous condition of probably recessive deleterious factors in the case of intraspecific pollination in alfalfa (5). In this species abortion was observed to be five times more frequent after self- than after cross-pollination.

The premature enlargement of the integument and the accompanying embryo sac degeneration seen in the smaller aborting ovules of *Asclepias syriaca* closely parallel the conditions associated with seed collapse in alfalfa. Here abortion of ovules in the free-nucleate endosperm stage was clearly distinguished by a multiplication of cell layers associated only with later stages of embryogeny in normal ovules. Although in most cases the endosperm showed evidence of disintegration, there were cases where no visible abnormality of the embryo sac could be noted. Nevertheless, in such ovules, the integument was definitely hyperplastic, indicating that abortion would doubtless soon follow. From such observations it may be concluded that the excessively rapid integument growth is the earlier morphological abnormality and that probably the unhealthy condition of the embryo sac is a result of the former activity.

Only in aborting ovules containing advanced multicellular endosperm and embryo was the zone of collapsed tissue about the embryo sac seen. Whether collapse of these seeds also can be attributed to tissue competition is less certain. The integument of successful seeds at a comparable stage is also extensively developed. Whether, in ovules destined to abort, development was initiated earlier than is normal cannot be decided from the material available. If so, it may be that the endosperm in spite of a slower growth rate was sufficiently vigorous to reach an advanced stage. The food available may have been so much diverted to the premature integument growth that, to survive, the endosperm was forced to absorb the contents of adjacent cells. Possibly also the collapse of the inner region of the integument represents the ordinary process of seed maturation occurring prematurely with respect to endosperm and embryo growth.

It is, however, also possible that an interruption of the food supply to potentially normal seeds has occurred and in an attempt to survive the endosperm has drawn upon the adjacent integument, causing the collapse of these cells. No morphological evidence of the cause of such an interruption could be detected. A general starved appearance of integument, funiculus, and placenta prevailed but neither block in the vascular tissue nor chalazal abnormality was apparent.

### Acknowledgments

The writer wishes to express his sincere gratitude to Dr. Margaret Landes and Dr. H. A. Senn for their advice during the course of the study and in the preparation of the manuscript.

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# INVESTIGATIONS ON RUBBER-BEARING PLANTS

## IV. CYTOGENETIC STUDIES IN *ASCLEPIAS* (TOURN.) L.<sup>1</sup>

BY RAYMOND J. MOORE<sup>2</sup>

### Abstract

The chromosome number of eight species and varieties of *Asclepias* (*A. syriaca*, *A. incarnata* and variety *pulchra* and forma *albiflora*, *A. Sullivantii*, *A. tuberosa*, *A. curassavica*, *A. speciosa*) was found to be  $2n = 22$ .

Various interspecific pollinations were attempted without success. A cross of *A. incarnata* with forma *albiflora* was successful.

Possible natural hybrids between *A. syriaca* and *A. speciosa* and between *A. incarnata* and var. *pulchra* and forma *albiflora* are described. Two unusual chimaeral plants of *A. incarnata* that produced several stems of forma *albiflora* are described.

### Introduction

In the course of recent wartime investigations of the possible economic uses of the genus *Asclepias*, chiefly the species *A. syriaca* L. and *A. incarnata* L., collections of these two and of several other species were assembled. As a preliminary to breeding work, the chromosome number of all the species and varieties was determined. Numerous attempts at intraspecific crosses of *A. syriaca* were made to work out a technique for artificial pollination of the curiously modified *Asclepias* flower. In addition, interspecific pollinations, involving all of the species available, were undertaken with a view to obtaining a hybrid that would surpass the native species in either latex or floss production.

### Materials and Methods

Seed was collected from native plants of *A. syriaca*, of *A. incarnata*, and of *A. Sullivantii*. Seed of other species was obtained from various Botanic Gardens. Plants were grown in plots at the Dominion Arboretum and Botanic Garden, Ottawa.

Herbarium specimens from these cultures are to be found in the Herbarium of the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa.

Chromosome counts were made from fresh smears of growing leaves and root tips fixed in Carnoy's mixture, made up as follows:

- 3 parts chloroform,
- 2 parts 95% ethanol,
- 1 part glacial acetic acid.

<sup>1</sup> Manuscript received March 12, 1946.

Contribution No. 852 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Earlier papers in this series were published as follows: I. The propagation of *Taraxacum kok-saghyz* Rod. by means of leaf cuttings. *Botan. Gaz.* 107(2): 260-267. 1945. II. Carbohydrates in the roots of *Taraxacum kok-saghyz* Rod. *Can. J. Research, C*, 24: 47-53. 1946. III. Development of normal and aborting seeds in *Asclepias syriaca* L. *Can. J. Research, C*, 24: 55-65. 1946.

<sup>2</sup> Agricultural Assistant.

The tissue was then stained in 45% aceto-orcein, heated, and flattened on the slide.

For one species, *A. curassavica*, root tips fixed in Allen's Bouin fluid, embedded, sectioned, and stained with Gentian violet were studied as well.

### Cytological Observations

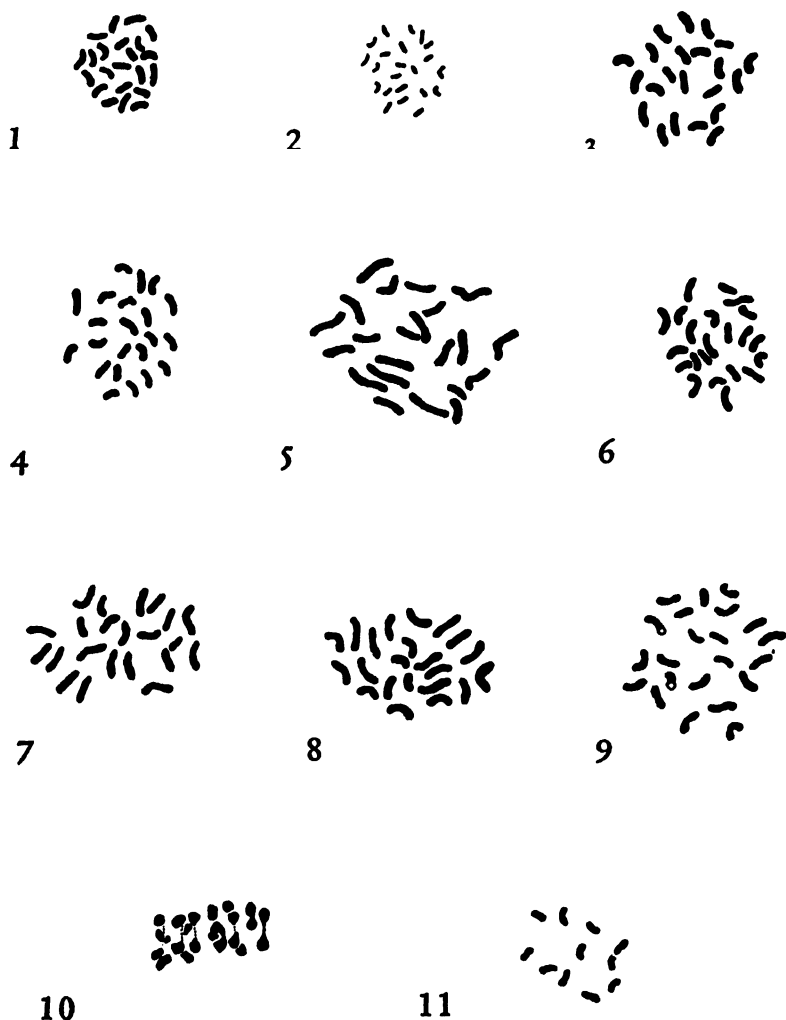
The diploid chromosome number of six species and two subspecific forms of one of these species was found to be 22. These counts, with previously reported determinations, are listed in Table I.

TABLE I  
CHROMOSOME NUMBERS IN *Asclepias* (TOURN.) L.

Species	<i>n</i>	<i>2n</i>	Author
<i>curassavica</i> L.		22	Moyer, 7
<i>curassavica</i> L.		22	Moore
<i>incarnata</i> L.		22	Moyer, 7
<i>incarnata</i> L.		22	Moore
<i>incarnata</i> L. forma <i>albiflora</i> Heller		22	Moore
<i>incarnata</i> L. var. <i>pulchra</i> (Ehr.) Pers.		22	Moore
<i>latifolia</i> Raf.		22	Moyer, 7
<i>salicifolia</i> Lodd.		22	Moyer, 7
<i>speciosa</i> Torr.		22	Moore
<i>Sullivantii</i> Engelm.	ca. 5		Frye, 5
<i>Sullivantii</i> Engelm.		22	Moore
<i>syriaca</i> L.	10		Strasburger, 11
<i>syriaca</i>	12		Stevens, 10; Gager, 6; Finn, 2
<i>syriaca</i>		22	Eichhorn and Franquet, 1
<i>syriaca</i> L.		24	Moyer, 7
<i>syriaca</i> L.	11	22	Moore
<i>tuberosa</i> L.	5		Frye, 4
<i>tuberosa</i> L.		22	Moore
<i>tuberosa</i> L. var. <i>sulfurea</i> L.		22	Moyer, 7
<i>verticillata</i>	ca. 8		Frye, 5

The chromosomes varied in length, in the preparations used, from 1 to 2.5  $\mu$ . It is not possible to recognize with certainty any of the homologous chromosomes although two or three pairs are somewhat longer than the others. Moyer (7) remarked that there was a noticeable difference in the morphology of the chromosomes of the different species. His figures show the chromosomes of *A. curassavica* to be smaller than those of five other species (see Table I). In the present investigation it was noted that the chromosomes of this species, in both root tips and leaf smears (Figs. 1, 2) were smaller than those of the other species examined. Differences between the chromosomes of the latter species were very slight. Some variation in morphology is very likely due to the physiological condition of the tissue and the rate at which mitoses have been taking place. For this reason it is felt that further comment on this variation is not justified.

In view of the disagreement (Table I) over the chromosome number in *A. syriaca* L. (*A. cornuti* Decaisne) special attention was paid to this species. Plants grown from seed collected from eight roadside stations about Ottawa, Ont. were examined but only the diploid number of 22 was found (Fig. 10). In Fig. 11 is shown the haploid number of 11 at II meiotic metaphase while in Fig. 10 are seen the 11 bivalents of I metaphase. Several additional counts of  $2n = 22$  were made by Dr. Margaret Landes, Division of Botany and Plant Pathology, Department of Agriculture.



All figures were drawn at a magnification of 2000 except Fig. 10,  $\times 1700$ . FIG. 2 shows somatic chromosomes from root-tips; Figs. 1 and 3 to 9 show the diploid chromosomes from leaf tissue.

1. *A. curassavica* L. 2. *A. curassavica* L. 3. *A. incarnata* L. 4. *A. incarnata* forma *albiflora* Heller. 5. *A. incarnata* variety *pulchra* (Ehr.) Pers. 6. *A. speciosa* Torr. 7. *A. Sullivantii* Engelm. 8. *A. tuberosa* L. 9. *A. syriaca* L. 10. *A. syriaca* L. I meiotic division, 11 bivalents. 11. *A. syriaca* L. II meiotic division,  $n = 11$ .

## Hybridization

### *Technique*

As is well known, the *Asclepias* flower is highly modified for insect pollination. The female parts, two separate ovaries, are almost entirely enclosed by a tube formed by the flattened filaments of the five stamens. Pollen is developed in flat club-shaped sacs (pollinia) of which there are two in each stamen. A thin tough band (retinaculum) fastens the pollinia in pairs, one member from each of two adjacent stamens. The upper portion of the styles of the two ovaries are united into a flat round stigma disk. Spaced at equal intervals around the margin of the lower surface of the disk are five stigmatic areas. Each stigmatic surface is thus located within a chamber, the outer walls of which are formed by portions of two adjacent flattened filaments, and is accessible to pollination only through a slit-like passage between the edges of the two filaments. By means of the retinacula, pollinia are readily entangled on the legs of insects gathering nectar and cross-pollination is effected by the insertion of one or more pollinia into the stigmatic chambers of the flower that the insect visits next.

In artificial pollinations, the pollinia were withdrawn from their pouches by means of a needle or fine pointed forceps and inserted into the chambers with an inward and upwards motion. Care was taken to cause as little injury as possible to the filament wings covering the entrance to the chamber. It was found after some preliminary attempts that no success was attained unless at least two adjacent chambers were pollinated with at least one pollinium each. From the arrangements of the stigmatic surfaces relative to the free styles, it appears that a passageway for pollen tubes to the same ovary will exist from four of the five possible pairs of adjacent stigmas. It was estimated that a pollinium of *A. syriaca* contains approximately 250 pollen grains. The greatest number of mature seeds obtained from a single pod was 243. It appears, therefore, that germination of a very high percentage of the pollen grains is required to fertilize all of the ovules present. Probably all of the grains in a sac do not germinate, or, if they do, cannot reach the stigmatic surface. It may be that pollen tubes from two stigmas are needed to fertilize a sufficient number of ovules to cause the ovary to remain attached to the stem.

Mature but unopened buds were used in artificial pollinations. The clusters were protected from subsequent insect pollination by paper bags. Usually only three to five flowers in an umbel were manipulated, the others were removed.

Following this technique a considerable number of intraspecific cross-pollinations of *A. syriaca* were successful.

The following interspecific hybridizations were attempted. The number of flowers crossed was relatively small. The approximate total number is indicated after each pollination. In all cases the female parent is named first. Pollinations in which some ovaries enlarged but aborted before maturity are indicated by an asterisk.

- A. syriaca* × *A. tuberosa* (50)  
*A. syriaca* × *A. incarnata* (28) and *A. incarnata* f. *albiflora* (6)  
*A. syriaca* × *A. speciosa* (10) and reciprocal (15)  
*A. speciosa* × *A. tuberosa* (20)  
*A. Sullivantii* × *A. syriaca* (15)  
*A. Sullivantii* × *A. speciosa* (7)  
*A. Sullivantii* × *A. tuberosa* (9)\*  
*A. incarnata* × *A. incarnata* v. *pulchra* (20) and reciprocal (30)  
*A. incarnata* × *A. incarnata* f. *albiflora* (20) and reciprocal (12)  
*A. incarnata* v. *pulchra* × *A. incarnata* f. *albiflora* (6) and reciprocal (20)  
*A. curassavica* × *A. syriaca* (6)\*  
*A. curassavica* × *A. tuberosa* (9) and reciprocal (4)  
*A. curassavica* × *A. incarnata* v. *pulchra* (4)  
*A. curassavica* × *A. incarnata* f. *albiflora* (5)  
*A. curassavica* × *A. speciosa* (4)\* and reciprocal (6)\*

Mature pods were obtained only in the cross *A. incarnata* × *A. incarnata* f. *albiflora*. It is felt that difficulties of manipulating the smaller flowers of *A. incarnata* were largely responsible for failure to obtain seed of the *A. incarnata* variety crosses.

After certain of the above cross-pollinations, as indicated, definite enlargement of the ovary was observed. The pod however did not mature and only small shrivelled seeds were found within. It is possible that fertilization occurred in these crosses but the seed aborted before maturity.

Seed gathered from *A. speciosa* plants in experimental plots in which *A. syriaca* also was growing produced plants that appeared to be hybrids between these species. Probably insect pollination was successful in effecting this cross. These possible hybrids showed intergradations in the leaf, flower, and stem characters of the two species.

Leaves of *A. syriaca* vary considerably in form and size—from almost round to linear-ovate and from 7 to 20 cm. in length—while those of *A. speciosa* are cordate-oval or oblong, generally smaller and covered with a silvery bloom, due to a fine pubescence. Typical *A. syriaca* plants reach 9 to 12 dm. in height, while those of *A. speciosa* are generally about 6 dm. The two species are separated most easily by their floral characters, especially the length of the hood. Hoods of *A. speciosa* are much longer (9 to 15 mm.) than those of *A. syriaca* (less than 7 mm.).

The hybrids could be classed as “*syriaca*-like *speciosa*” and as “*speciosa*-like *syriaca*”. The former type resembled *A. syriaca* in regard to leaf size and shape and stem height, but the leaves bore the pubescence of *A. speciosa*. However, judging by the hood length (9 to 10 mm.), this type was to be placed in the latter species.

The “*speciosa*-like *syriaca*” plants were similarly placed within the species *syriaca* on the basis of hood size. They resembled *A. speciosa* in leaf shape and texture and in the lesser stem height.

Cross-pollinations of these possible hybrids with more typical individuals of each species were attempted; the results are shown in Table II.

TABLE II

RESULTS OF ARTIFICIAL POLLINATIONS OF TYPICAL *A. speciosa* AND *A. syriaca* WITH INTERGRADING FORMS OF BOTH SPECIES

Pollination	No. fls. pollinated	No. pods obtained
<i>A. syriaca</i> ( <i>speciosa</i> -like) $\times$ <i>A. speciosa</i>	7	1
<i>A. speciosa</i> ( <i>syriaca</i> -like) $\times$ <i>A. syriaca</i>	4	0
<i>A. speciosa</i> ( <i>syriaca</i> -like) $\times$ <i>A. syriaca</i> ( <i>speciosa</i> -like)	4	2
<i>A. speciosa</i> $\times$ <i>A. syriaca</i> ( <i>speciosa</i> -like)	11	2
<i>A. speciosa</i> ( <i>syriaca</i> -like) $\times$ <i>A. speciosa</i>	3	0

Similar supposed hybrids between these species were mentioned by Stevens (9). Moreover, this author was successful in obtaining seven mature pods from approximately 90 flowers of *A. syriaca* pollinated artificially with pollen of *A. speciosa*.

Possible hybrids of *A. incarnata* with the variety *pulchra* and the form *albiflora* were grown from seed gathered from plants in experimental plots containing these three types.

*Asclepias incarnata* variety *pulchra* (Ehr.) Pers. is distinguished from the species by its broader leaves and greater degree of pubescence on stem and leaf. The stem of the species is glabrous or bears only a slight line of hair. Further, variety *pulchra* has paler flowers, dull rose in colour, in contrast to the bright rose of the species.

These apparently hybrid plants showed the *pulchra* flower colour associated with the relatively glabrous stem and the leaf characters of the species. Varying degrees of stem pubescence also occurred. The association of the *incarnata* flower colour with the pubescence and leaf shape of the variety has not been seen. Seed of a single pod frequently produced a population including individuals with *pulchra* flower colour and glabrous stem and other plants completely typical of the species.

The form *albiflora* differs from the species only in pigmentation. The flowers of the former are white and pigment is wholly lacking in stems and flower peduncles and pedicels. Apparent hybrids between this form and the species also were found. Seed of a single pod of an *A. incarnata* plant sometimes produced plants that appeared typical and others that lacked pigment and were identical with the form *albiflora*.

The cross *A. incarnata* f. *albiflora*  $\times$  *A. incarnata* was made artificially. All individuals (11) of the  $F_1$  generation showed the bright rose flower colour and rose-purple stem pigmentation of *A. incarnata*. Presumably flower colour and stem pigmentation is genetically dominant to the lack of these characters.

This conclusion was further supported by the ratio between the number of white and of coloured individuals in the natural hybrid populations referred to above.

From these observations it seems probable that the three forms of this species can be hybridized and that difficulties in technique were the cause of the failure of some of the cross-pollinations attempted.

Two plants were observed that apparently were chimaeras of tissue of *A. incarnata* and of the form *albiflora*. In these, several pigmented stems bearing coloured flowers and, as well, some unpigmented stems with white flowers arose from a single root. Examinations of leaf smears from the two kinds of stems revealed that the chromosome number was the same in both,  $2n = 22$ . It seems therefore that the colour difference was caused by a gene mutation rather than by either a duplication or loss of a whole chromosome in either form. These plants were grown from seed collected from plots of *A. incarnata* f. *albiflora*. It is quite possible that the seed plants had been crossed by insect activity with nearby plants of *A. incarnata*. In this case it would be expected that the seed would produce heterozygous plants showing the dominant characters of the species. It is therefore impossible to say whether the mutation produced *albiflora* stems from pigmented tissue or the reverse. Only four plants survived to maturity in this plot, the other two being phenotypically *A. incarnata*. A view of the root of one of these plants, showing three unpigmented and four pigmented stems, is seen in Fig. 12.

### Self-fertility

Self-pollination of *A. syriaca* and of *A. incarnata* was attempted but always without success. That self-fertile plants of both species exist has been reported by Plotnikova (8) and Stevens (9) for *A. syriaca*, and for *A. incarnata* by Fischer (3).

### Acknowledgments

A considerable part of the intraspecific pollinations of *A. syriaca* was done by Miss M. K. Osler, summer assistant. The writer wishes to express his gratitude for this assistance. He is indebted also to Dr. Margaret Landes and Dr. H. A. Senn for assistance and criticism in the preparation of the manuscript. Determinations of *A. syriaca* and *A. speciosa* were made with the aid of a new key prepared by Mr. M. N. Zinck.

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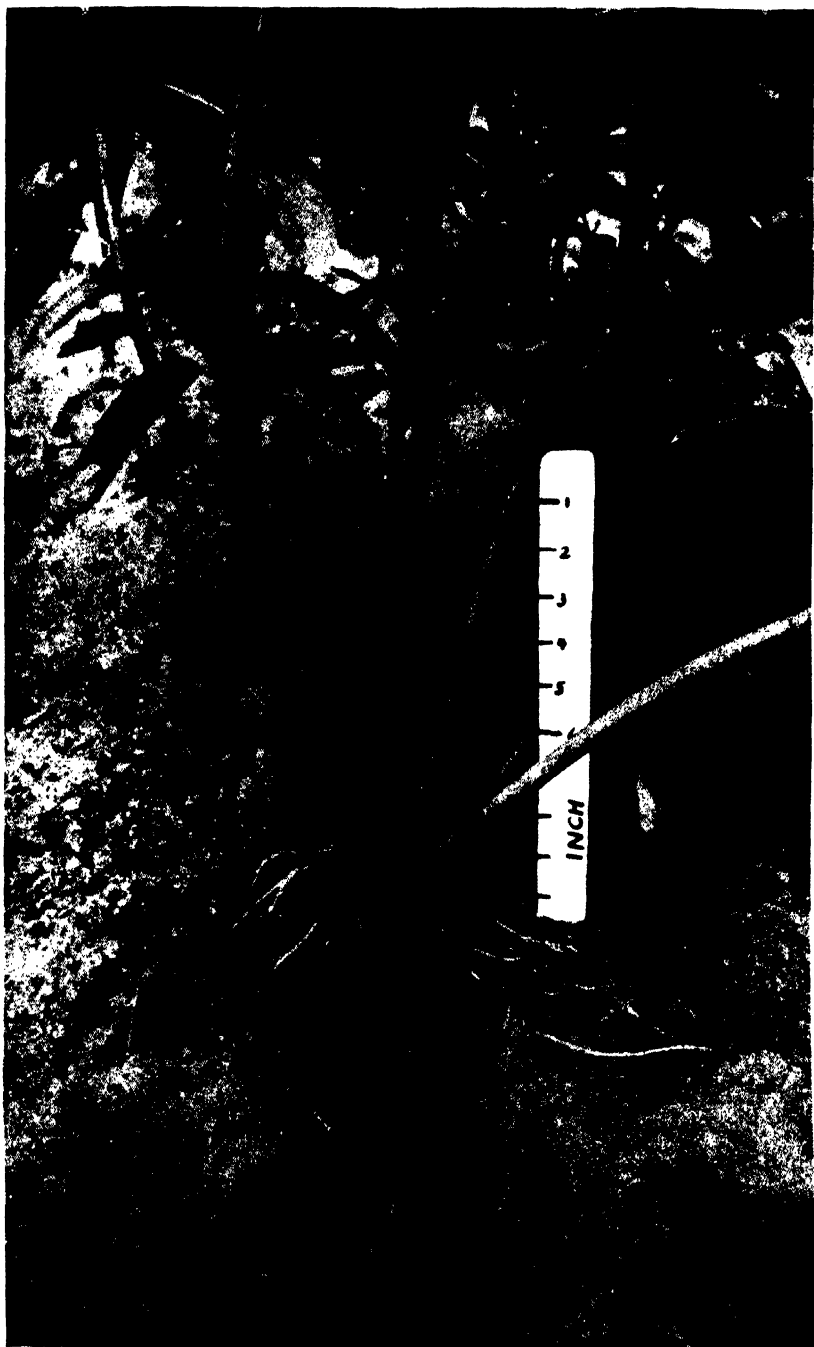


FIG. 12. Root and lower part of stems of a chimaeral plant of *Asclepias incarnata* L. showing three unpigmented and four pigmented stems.





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## NOTES ON SEED-BORNE FUNGI

### IV. *ACREMONIELLA*, *CHLAMYDOMYCES*, AND *TRICHOCLADIUM*<sup>1</sup>

BY J. W. GROVES<sup>2</sup> AND A. J. SKOLKO<sup>3</sup>

#### Abstract

Two species of *Acremoniella*, *A. atra* (Cda.) Sacc. and *A. verrucosa* Togn., one species of *Chlamydomyces*, *C. palmarum* (Cke.) Mason, and one species of *Trichocladium*, *T. asperum* (Cda.) Harz, have been isolated from agricultural seeds and are described and illustrated. These fungi do not appear to be of serious pathological significance.

#### The genus *Acremoniella*

The genus *Acremoniella* was erected by Saccardo (12) as a segregate from *Acremonium* Link and was said to differ from *Acremonium* in having coloured spores. Seven species were listed of which the second, *A. atra* (Cda.) Sacc., should be regarded as the type. Mason (11) has pointed out that the first species, *A. fusca* (Kze. & Schm.) Sacc., has not been recognized with certainty since, and that *A. atra* was the only species that Saccardo had studied himself. *A. atra* is the commonest and best known species and its recognition as the type of the genus would fix the generic name in the sense commonly used.

The genus is characterized by the large, brown, one-celled, ovoid to subglobose spores borne singly on the tips of pointed, septate, branched conidio-phores. In the terminology used by Mason (11) these spores are considered to be aleuriospores, or essentially terminal chlamydospores. He also described phialospores but, in the light of later knowledge of sexuality in the ascomycetes, these should probably be interpreted as spermatia. The perfect stage has not been established, but Mason suggested that it might prove to be a *Melanospora*. No perfect stage has appeared in any of our cultures.

We have isolated two species of *Acremoniella* from seeds, *A. atra* (Cda.) Sacc. and *A. verrucosa* Togn.

***Acremoniella atra*** (Corda) Sacc. Syll. Fung. 4:302. 1886.

This is the best known species of the genus, and the concept of the species appears to be clear-cut but it raises an interesting problem in nomenclature.

The specific name is based on *Acremonium atrum* Corda published in 1837; consequently the validity of Saccardo's combination, *Acremoniella atra*, rests on whether or not Saccardo correctly identified his fungus as *Acremonium atrum* Corda. Mason was unable to locate any type specimen of *A. atrum* Cda. and argued that if no Corda specimens existed, it was possible to accept

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Saccardo's determination and recognize Corda's specific name as valid. This has the advantage of retaining a well-known name but raises the question as to what is to be considered the type of the species.

The International Rules provide that a figure may be taken as the type when no specimen exists. Corda's figure in Pl. 3, Fig. 68, of the *Icones*, labelled *Acremonium atrum*, illustrates a fungus with spores somewhat similar in shape to those of *Acremoniella atra* as commonly conceived, but borne on somewhat different conidiophores. In this figure the conidiophores are long, unbranched, with rather numerous, close septa, and tapering gradually and evenly from the base to the tip. This is in contrast to the rather sparingly septate conidiophores of *Acremoniella*, which branch frequently at right angles or nearly so, and usually taper gradually near the base and more abruptly toward the tip. It would appear that Corda's figure could only be accepted as the type of Saccardo's fungus by assuming that Corda drew the conidiophores inaccurately.

However, not only Corda's figure but also his description does not fit our fungus. His choice of the specific name "*atra*" would imply that the fungus was black, and Mason (11) pointed out that Corda's description stated that the thallus and spore contents were very black, but *Acremoniella atra* in Saccardo's sense is not black.

Thus there is good reason to believe that Saccardo's identification of his fungus as *Acremonium atrum* Corda was incorrect, and that consequently the use of the specific name "*atra*" for this fungus is not permissible. According to the synonymy proposed by Mason (11), the next available name is *Monosporium acremonioides* Harz, published in 1871. A new combination in *Acremoniella* based on this species would, therefore, probably provide the correct name for our fungus, and Harz's material would become the type. Unfortunately, no material of *Monosporium acremonioides* is available at present to establish this identification, and to create a new combination now would be merely substituting one guess for another. Furthermore, since Mason's paper was published, at least part of Corda's herbarium has been discovered in Prague and it is possible that a Corda specimen may yet come to light that will establish beyond question the identity of *Acremonium atrum*.

Until such time as the Corda and Harz specimens can be further studied we have, therefore, decided to use the well-known name *Acremoniella atra* for this fungus. Probably the name will have to be changed, but this should only be done after comparison with authentic material of *Monosporium acremonioides*. In the meantime, the use of a well-known name, even though incorrect, seems preferable to creating a new combination based merely on a supposition.

*A. atra* appears to be widely distributed on various substrata and is fairly well-known. Mason (11) described the growth in culture quite adequately, and gave a list of synonyms. We have never isolated it directly in pure culture from seeds but it has been invariably associated with *Alternaria tenuis* auct. It was easily obtained in pure culture by picking off the large spores.

The spores are golden brown or cinnamon brown, one-celled, ovoid to subglobose, usually smooth walled, sometimes with a slight wrinkling of the exospore,  $(19)\text{--}22\text{--}28\text{--}(32) \times (16)\text{--}18\text{--}24\text{--}(26)\mu$  (Figs. 1, 5). The conidiophores are hyaline, rather sparingly septate, simple or branching at right angles or nearly so, straight or slightly curved, variable in length,  $4\text{--}8\mu$  in diameter at the base, tapering to a slender tip where the spores are borne. The microconidiophores are hyaline, septate, variable in length, aspergilliform with a terminal vesicle about  $12\mu$  in diameter bearing flask-shaped phialides about  $4\mu$  in diameter at the base and  $6\mu$  in length. The microconidia are hyaline, ovoid to globose,  $1.5\text{--}2.0\mu$  in diameter, borne in chains.

It has been isolated from the following seeds: *Allium cepa* L. (onion), *Beta vulgaris* L. (beet), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca rubra* L. (red fescue), *Lolium perenne* L. (rye grass), *Pastinaca sativa* L. (parsnip), *Pisum sativum* L. (peas), *Raphanus sativus* L. (radish), *Trifolium hybridum* L. (alsike clover), *Zea mays* L. (corn).

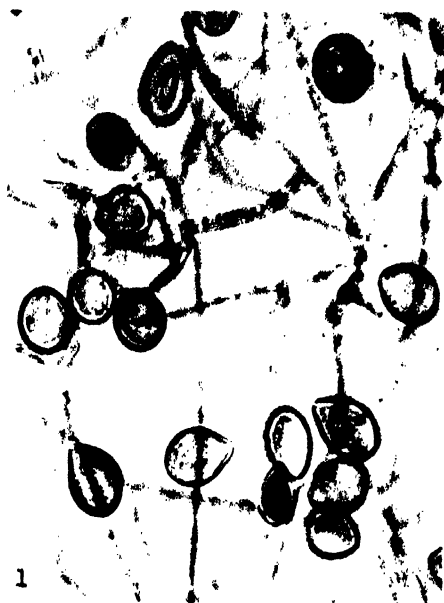
It has been isolated from seeds from Ontario, British Columbia, Denmark, and Holland.

As noted above, we have always found *A. atra* associated with *Alternaria tenuis* auct. when isolated from seeds. Under these conditions it grows rapidly, producing a luxuriant development of aerial mycelium and abundant spores. When grown in pure culture, however, it grows much more slowly, produces less mycelium and fewer spores.

In order to find out whether the difference in growth rate was due to the presence of the *Alternaria*, Petri dishes were inoculated at opposite sides with cultures of *Acremoniella* and *Alternaria*. Malt extract agar and potato dextrose agar were used. Growth was uniform in both fungi until the colonies met, when a pronounced stimulation of growth occurred in the *Acremoniella*. From the point of contact of the two mycelia, the hyphae of the *Acremoniella* quickly spread over and through the *Alternaria* colony. There was no corresponding stimulation of the *Alternaria* and hyphae of the *Acremoniella* that did not actually come into contact with the *Alternaria* did not exhibit any stimulation. The same effect was observed when *A. atra* was grown in association with *Fusarium moniliforme* Sheldon and *Cephalosporium acremonium* Cda.

Mason (11) observed that *A. atra* would not grow in pure culture on Dox agar, but would grow in association with *Cephalosporium acremonium* Cda. We repeated this experiment and obtained the same result with an isolate of *C. acremonium* from corn, but with an isolate of *C. acremonium* from peas, which was morphologically indistinguishable from the corn isolate, it refused to grow.

FIG. 1. Microphotograph of *Acremoniella atra* (Corda) Sacc. FIG. 2. Microphotograph of *Acremoniella verrucosa* Togn. FIG. 3. Microphotograph of *Chlamydomyces palmarum* (Cooke) Mason. FIG. 4. Microphotograph of *Trichocladium asperum* (Cda.) Harz.





It is evident that the presence of other fungi may have a decided effect on the growth of *A. atra*, not only in the synthetic media such as Dox agar, but also in carbohydrate media such as malt extract agar, pea decoction agar, and potato dextrose agar. Nothing is known of the reason for this reaction but several interesting problems are suggested by it. The fungus is generally regarded as a saprophyte but it would be desirable to investigate the possibility that if it is pathogenic, its pathogenicity might also be affected by the presence of other fungi.

*A. verrucosa* Togn. Rend. ist. lombardo sci., 2 ser., 29 : 864. 1896.

This species can be distinguished from *A. atra* by the rough-walled, slightly smaller, and more globose spores. They are light brown, ovate to globose, one-celled, verrucose to tuberculate,  $17-26 \times 17-23\mu$  (Figs. 2, 6). The conidiophores are hyaline, septate, simple or usually branched at right angles or nearly so, variable in length,  $5-8\mu$  in diameter at the base, tapering to a slender tip on which the spore is borne. The microconidiophores are aspergilliform, hyaline, septate, not branched, variable in length, about  $5-6\mu$  in diameter, swollen at the tip into a vesicle  $10-13\mu$  in diameter on which flask shaped to ovoid phialides,  $4-5\mu$  in diameter at the base and  $4-10\mu$  in length, are borne. The microconidia are hyaline, one-celled, broadly ellipsoid to ovoid, catenate,  $2.5-3.0 \times 1.5-2.0\mu$ .

This species has only been isolated on two occasions, once from seed of *Pisum sativum* L. from Ontario and once from seed of *Taraxacum kok-saghyz* Rod. from the U.S.S.R.

Horne and Jones (7) who studied *A. verrucosa* and *A. atra* under the names *Eidamia tuberculata* and *E. acremonioides* respectively, considered that in addition to their morphological differences, they were distinct physiologically in the ability of *A. verrucosa* to utilize certain media that *A. atra* could not. Mason (11) questioned this because he found, from a study of the type culture of *Eidamia tuberculata*, that it behaved like a mixture of *A. atra* and *Cephalosporium* on Dox agar.

When grown on Dox agar in pure culture, our isolates of *A. verrucosa* also failed to grow. In association with *Alternaria tenuis* or *Fusarium moniliforme*, they grew and sporulated profusely, but with *Cephalosporium acremonium* growth was slight. This confirms Mason's findings that *A. atra* and *A. verrucosa* behave similarly on Dox agar.

### The Genus *Chlamydomyces*

The genus *Chlamydomyces* was erected by Bainier (1) and was based on a fungus that he described as *C. diffusus*. This fungus, collected on cow dung, was said to have the mycelium and conidiophores of *Acremoniella atra* but the spores of *Mycogone cervina*. He considered that it differed from *Mycogone* in its saprophytic rather than parasitic habit of growth and in its lack of a microconidial stage.



Later work has shown that the latter distinction does not hold. Hotson (8) reported microconidia in *Mycogone Ulmariae* Potebnia, which he considered to be a synonym of *Chlamydomyces diffusus*. Mason (10) also described microconidia in *Chlamydomyces* but thought the genus should be accepted and could be distinguished from *Mycogone* by the cuneate rather than subglobose basal cell of the spore, and by the lack of constriction at the septum. He was of the opinion that species of *Mycogone* were parasites of other fungi and that all records of *Mycogone* on flowering plants should probably be referred to *Chlamydomyces*.

The similarity between *Chlamydomyces* and *Acremoniella* has been apparent to all who have studied these species. They have the same type of microconidial fructification and similar growth habit, and differ chiefly in the two-celled spores of *Chlamydomyces*. There is some evidence that the small basal cell of the *Chlamydomyces* spore is in reality the tip of the conidiophore, and, if this is the correct interpretation, as seems probable, even this distinction breaks down. The two genera are undoubtedly very closely related. The perfect stage of *Chlamydomyces* is still unknown. Only the one species has been isolated from seeds.

***C. palmarum*** (Cooke) Mason. Annotated account of fungi received at the Imp. Bur. Mycol. List 2, Fasc. 1 : 37. 1928.

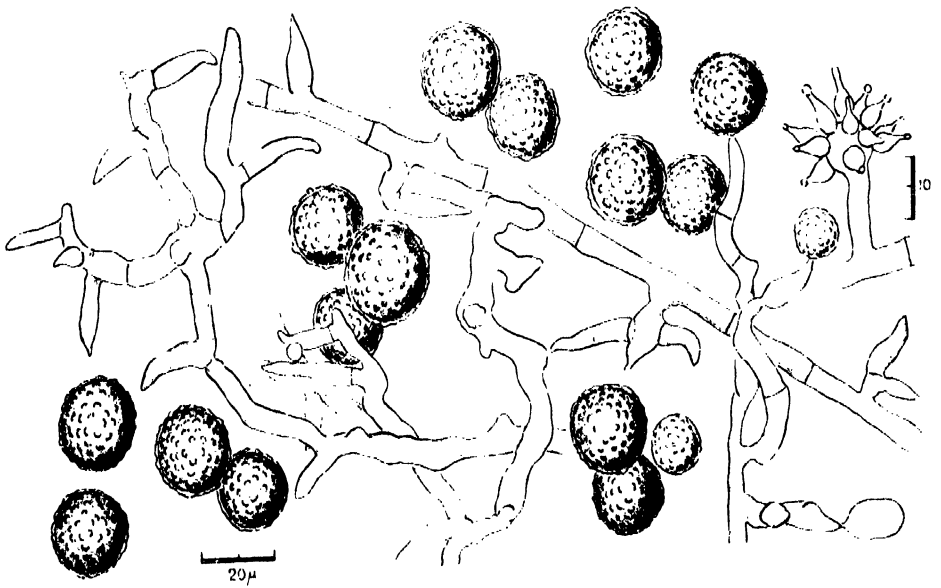
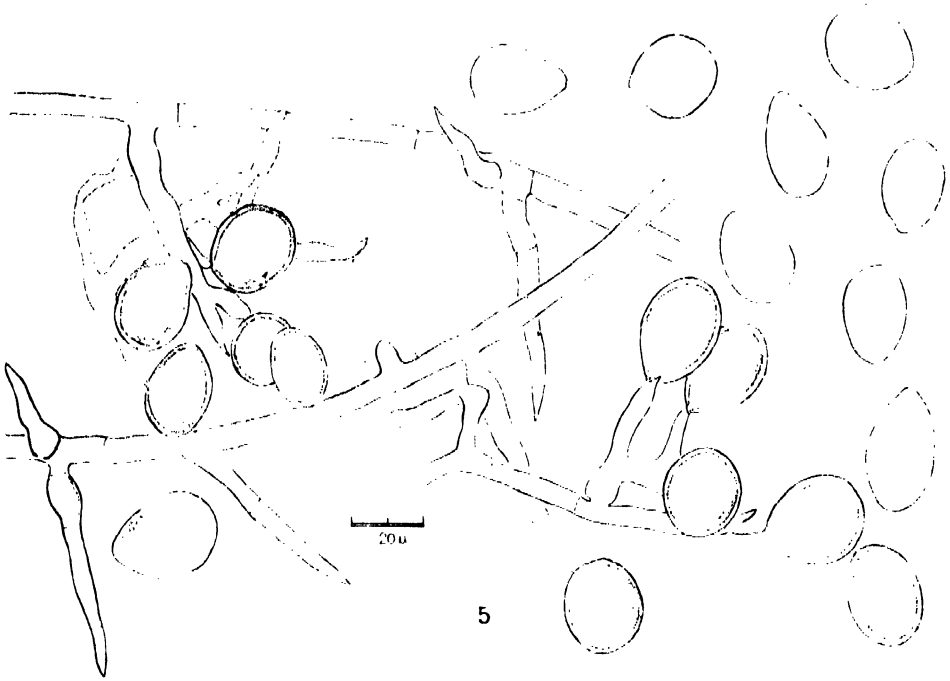
This species was originally described by Cooke (3) as *Trichobasis palmarum* on leaves of *Cocos nucifera* and was transferred to *Chlamydomyces* by Mason (10) after comparison of living cultures with authentic specimens in Kew Herbarium. Mason was unable to locate any type material of *C. diffusus* Bainier, the type of the genus, but there seems little doubt that it is the same fungus. Our isolates agree well with the descriptions and one was sent to Mr. Mason, who kindly confirmed the identification.

The spores are ovoid, narrowed toward the base, two-celled, not constricted at the septum, the upper cell yellow, verrucose, and much larger, the lower cell hyaline, smooth, cuncate, and much smaller,  $28-50 \times 22-40\mu$  (Figs. 3, 8). The conidiophores are hyaline, septate, simple or branched at right angles or nearly so, variable in length,  $7-10\mu$  in diameter at the base, tapering to a slender tip. The microconidiophores are hyaline, septate, simple, variable in length,  $4-5\mu$  in diameter, aspergilliform, the terminal vesicle  $8-12\mu$  in diameter and bearing flask-shaped phialides  $4-5\mu$  in diameter at the base and  $7-13\mu$  long, the phialides occasionally produced singly on the hyphae. The microconidia are hyaline, one-celled, oblong to ellipsoid,  $3-5 \times 1.5-2.0\mu$ , borne in chains.

It has only been isolated from seeds on two occasions, once from *Pastinaca sativa* L. (parsnip), and once from *Pisum sativum* L. (peas). The seed of both samples was produced in Ontario.

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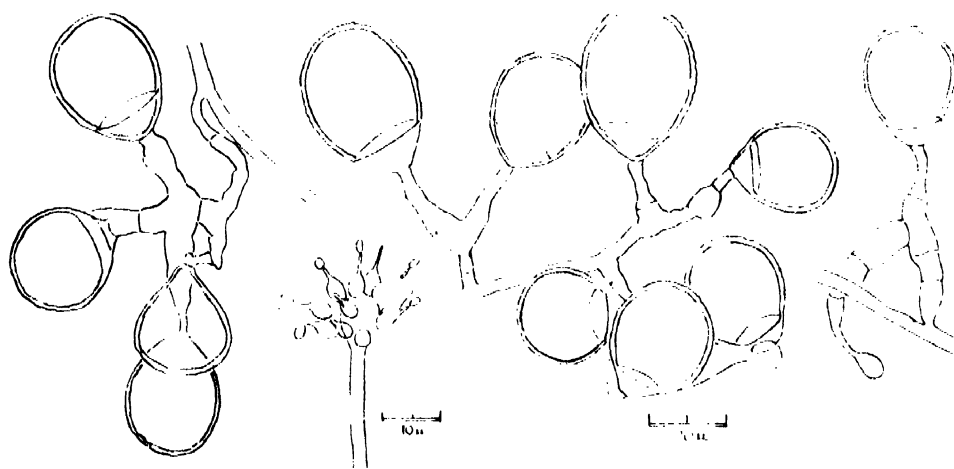
FIG. 5. *Acremoniella atra* (Corda) Sacc. FIG. 6. *Acremoniella verrucosa* Togn.  
FIG. 7. *Trichocladium asperum* (Cda.) Harz. FIG. 8. *Chlamydomyces palmarum*  
(Cooke) Mason.













This species sporulates well and produces a more luxuriant growth of mycelium on malt extract agar than either of the *Acremoniella* species.

On Dox agar some growth occurred but it was very markedly reduced as compared to the growth on malt. A few rather coarse hyphae were produced that grew in a straggling fashion over the plate with few branches or aerial hyphae and very scant spore production. When grown with *Cephalosporium acremonium* on the same medium there was some evidence of stimulation but it was slight. However, with *Alternaria tenuis* and *Fusarium moniliforme* it grew luxuriantly and produced abundant aerial mycelium and spores. The similarity of this behaviour to that of the *Acremoniella* species would further indicate a close relationship between them.

### The Genus *Trichocladium*

The genus *Trichocladium* was erected by Harz (6) based on a fungus that he considered to be *Sporidesmium asperum* Corda. The spores are two-celled, dark, rough-walled, borne singly on the tips of short conidiophores. It seems to be close to the older genus *Dicoccum* Corda, and some authors, Lindau (9), Clements and Shear (2), and Gilman (5), have considered it to be a synonym. However, Mason (11) has suggested that because the structure of the type of *Dicoccum* is obscure and Corda himself did not recognize *Sporidesmium asperum* as a species of his genus *Dicoccum*, it would be advisable to retain *Trichocladium* for the present. Only one species has been isolated from seeds.

***Trichocladium asperum*** (Cda.) Harz. Bull. soc. imp. naturalistes Moscou, 44 : 125. 1871.

On malt extract agar different isolates vary to some extent in colour and amount of aerial mycelium. They may be dark olive grey to iron grey or dark greenish olive to light yellowish olive, to olive yellow or olive ochre (Ridgway). The conidiophores are hyaline, simple or occasionally branched, septate, 3–4 $\mu$  in diameter, variable in length, 10–56 $\mu$  or more. The conidia are oblong-ellipsoid to clavate, at first hyaline, becoming brown and finally nearly black, two-celled, slightly constricted at the septum, the upper cell rounded, the lower cell narrowed toward the base, at first slightly smaller than the upper cell, later about the same size, tuberculate, (14)–16–22–(25)  $\times$  (10)–11–14–(15) $\mu$  (Figs. 4, 7). Occasionally the spores become three-celled and sometimes one-celled spores are formed. The latter are ovoid to subglobose and about the same size as a single cell of the normal two-celled spores. Frequently a small piece of the end of the conidiophore remains attached to the spore, appearing as a short pedicel. No microconidia have been observed.

It has been isolated from seeds of *Cucurbita Pepo* L. (pumpkin), *Daucus carota* L. var. *sativa* DC. (carrot), *Lactuca sativa* L. (lettuce), *Pisum sativum* L. (peas), and *Vicia faba* L. (broad beans). It has been isolated from seeds from British Columbia, Connecticut, and Missouri.



In his account of *T. asperum*, Harz (6) expressed some doubt as to the correctness of his identification of this fungus as *Sporidesmium asperum* Cda. He pointed out some discrepancies between Corda's description and figure but concluded that if the characters of both description and figure were taken, it was probably the same as his fungus. Dufour (4) described the growth in culture and the way in which the spores are formed. He was of the opinion that there was less difference between the figures of Corda and of Harz than between cultures of the same strain grown on sterilized orange juice and a sugar solution, and that Harz's identification was probably correct. It would seem, therefore, that this name may be accepted as valid unless someone can show from an examination of Corda's material that *Sporidesmium asperum* was a different fungus.

This species exhibited no stimulation of growth when grown with *Alternaria tenuis*, *Cephalosporium acremonium*, or *Fusarium moniliforme*, and it grew quite well in pure culture on Dox agar. The appearance of the cultures and manner of growth is very different from cultures of *Acremoniella* and *Chlamydomyces*. In spite of the superficial similarity of dark, rough-walled spores, it seems probable that when the perfect stages of these forms are eventually discovered, *Trichocladium* will prove to be not closely related to the other two genera.

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# TETRAPLOID *TARAXACUM KOK-SAGHYZ*

## II. CHARACTERS OF $F_1$ PLANTS GROWN IN POTS<sup>1</sup>

BY M. W. BANNAN<sup>2</sup>

### Abstract

$F_1$  tetraploids grown in pots had larger but fewer leaves and inflorescences than control diploids. Increase in size of the organs was offset by reduction in number, so that leaf area was no greater and total number of florets per plant was much lower in tetraploids than in diploids. Fertility varied inversely with genetic relationship and was much reduced in the tetraploids. No significant differences were noted in root size. The tetraploids grew slightly more slowly than diploids but for a longer period, and, on the whole, plants with the largest rosettes developed the biggest roots. Sibling tetraploids exhibited less deviation from the mean than randomized plants in such characters as number, size, and shape of leaves, production of heads, and weight of roots. Leaf characteristics provided the clearest evidence of an inheritance mechanism. The data on root weight were less conclusive.

### Introduction

In a previous publication (2) the production of tetraploid kok-saghyz by the colchicine technique was described. Certain characteristics of the  $4n$  plants were discussed, but since the majority of the colchicined plants were chimaeras, it was pointed out that the critical determination of the effects of tetraploidy would have to be made from the second colchicine ( $C_2$ ) or first filial ( $F_1$ ) generation. Such plants are described in the present paper and comparison made with diploids grown in parallel series. It should be noted that the descriptions refer to plants grown in pots. Observations on  $2n$  and  $4n$  kok-saghyz cultivated under agronomic conditions do not come within the scope of this paper.

### Methods

For comparative studies five series of  $F_1$  tetraploids and diploids were established, differing in origin of seed and conditions of growth. These series were designated as  $A^1$ ,  $B^1$ ,  $C^1$ ,  $D^1$ , and  $E^1$ , but were not necessarily derived from the series of corresponding designation ( $A$  to  $E$ ) in the parental generation already described (2). The seed was obtained in two ways. The first lot of  $4n$  seed was procured by random, unbagged, and unrecorded crossing of tetraploids in parental Series  $C$ . The second lot was obtained a few weeks later by controlled, bagged crossing of described and recorded plants in Series  $A$ ,  $B$ , and  $C$ . The roots of these parental plants were later weighed and the rubber content of many was determined. The first lot of diploid seed was collected at random from parental Series  $C$ , the plants of which were colchicined, selected on the stomatal criterion as possible tetraploids, but produced predominantly diploid inflorescences. The second lot of diploid seed was a

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random mixture from untreated field diploids. The origin and handling of the several series are summarized in Table I.

The growing season of 1945 was unusual. Owing to persistent, deep snow cover, the ground was never frozen during the winter. The snow disappeared

TABLE I  
ORIGIN AND TREATMENT OF  $F_1$  DIPLOIDS AND TETRAPLOIDS

Series	Purpose	Origin of seed		Conditions of growth	
		2n	4n	Early growth	Subsequent growth
A <sup>1</sup>	Comparison under suppressed growth of diploids and tetraploids both derived from colchicined parents	Random crosses of colchicined parents	Random crosses	Germinated Aug. 1944. Set out in flats, and later transferred to 4-in. pots	Plunged outside and exposed to ground frost. Returned to greenhouse in Nov. Little winter growth. Repotted in 6-in. pots Mar. 1945. Retained in greenhouse all summer. Growth meagre
B <sup>1</sup>	Comparison under optimal growth of plants similar to A <sup>1</sup>	As above	As above	As above	Plunged outside and there overwintered. Returned to greenhouse Mar. 1945 and repotted in 9-in. pots. Growth rapid and luxuriant. Largest and most floriferous plants occurred here
C <sup>1</sup>	Comparison under intermediate growth conditions of plants similar to A <sup>1</sup>	As above	As above	As above	Plunged outside and there overwintered. After repotting in 8- or 9-in. pots were left to grow out of doors, spring and summer 1945. Growth moderate, flowering later and less than B <sup>1</sup> . Reaction to summer dormancy more severe
D <sup>1</sup>	To determine whether $F_1$ tetraploids gave evidence of inheritance mechanism for vegetative and reproductive characters	As above	Recorded crosses between parents with wide range of leaf type, root size, and rubber content	Germinated Mar. 1945. Set out directly in 2-in. pots, then transferred to 8-in. pots	Plunged outside. Growth good, but of one season only
E <sup>1</sup>	To function as control and to permit cross comparisons with D <sup>1</sup> of colchicined diploids, random diploids, random tetraploids, and recorded tetraploids	Random crosses of untreated field plants	Randomized seed of recorded crosses	As above	As above

during unseasonably warm weather in April. May was excessively wet and cool, severely retarding growth out of doors. Moisture was superabundant throughout June. When drier weather came in August a marked summer dormancy was manifested in the field from which later recovery was but slight. All plants were unearthed in October, 1945. The leaves were removed and the roots washed and weighed. Some were dried and stored for rubber analysis, others were repotted for future crossing.

### Germination and Seedling Development

Germination of the achenes from colchicined parental diploids and tetraploids was retarded and this effect was attributed to the choice of cold and humid rather than warm and dry conditions of winter storage. The germination of seed from untreated field diploids stored at room temperature seemed unaffected. Under the retarded conditions no consistent differences were observed between random  $2n$  and  $4n$  seed in either rate or amount of germination. In the growth of the seedlings it was noted that the diploids produced more numerous, slightly longer, but definitely narrower leaves.

The controlled  $4n$  crosses exhibited wide differences in the rate and amount of germination. The achenes from one seed parent in particular were notable for rapid germination. In certain other cases germination was tardy but ultimately high. Some parental individuals were good both as seed and pollen parents; others were good pollen parents but poor seed parents, or vice versa. Mean weight of the sound achenes varied from 0.52 to 0.98 mgm. in the crosses studied, but weight was not the major factor in determining germination or final size of the plant. Achenes from the upper half of the seed population weighed 0.71 to 0.98 mgm., germinated to 74%, produced rosettes with an average of 29 leaves at the peak of leaf production, and roots weighing 16.4 gm. Achenes from the lower half, weighing 0.52 to 0.71 mgm., germinated to 83% and developed plants of 28 leaves and roots of 19.1 gm.

### Leaves

#### *Number of Leaves*

Leaf production varied widely, as shown in Table II, but was, on the whole, markedly greater in  $2n$  than in  $4n$  plants. The first-year diploids (Series  $D^1$  and  $E^1$ ), at the point of maximum development, had 84% more green leaves than had the corresponding tetraploids, and in second-year plants (Series  $B^1$  and  $C^1$ ) the margin was 64%. The differences in total leaf production were much wider than these values indicate, because of the more rapid turnover of leaves in the diploids. In this, as in other growth characteristics, the tetraploids appeared torpid as compared with the diploids.

The greatest leaf production occurred in Series  $B^1$  where the pots were largest and the growing season longest. Leaf development was greater also in second-year (Series  $B^1$  and  $C^1$ ) than in first-year (Series  $D^1$  and  $E^1$ ) plants, especially in the tetraploids. Among the latter, wide differences were noted between the various crosses. For instance, the  $F_1$  progeny of the  $31 \times 86$

developed rosettes with an average maximum of 40 leaves, whereas in the progeny of  $71 \times 296$  and  $296 \times 71$  the average numbers were 24 and 22.

### *Length of Leaves*

Employing rosette diameter as the criterion, leaf length was found to vary greatly between individuals, but Table II shows that at the peak of development mean differences between  $4n$  and  $2n$  plants lay within 11%. Except in

TABLE II

RANGE AND AVERAGE DIAMETER OF THE ROSETTES AND NUMBER OF GREEN LEAVES AT MAXIMUM DEVELOPMENT (1945)

Series	$2n$			$4n$		
	No. of plants	Diam. in in.	No. of leaves	No. of plants	Diam. in in.	No. of leaves
$A^1$	21	5-9 (7.2)	16-62 (39)	36	5-10 (7.5)	14-57 (34)
$B^1$	18	10-14 (12.0)	30-138 (75)	36	9-18 (12.6)	20-127 (48)
$C^1$	34	8-14 (10.1)	27-122 (52)	66	7-14 (9.5)	13-50 (30)
$D^1$	42	7-13 (9.2)	22-83 (45)	548	5-14 (8.8)	9-76 (29)
$E^1$	32	7-12 (9.3)	16-132 (60)	33	5-14 (8.4)	13-96 (28)

the suppressed Series  $A^1$ , the rosettes were wider in second-year (Series  $B^1$  and  $C^1$ ) than in first-year plants (Series  $D^1$  and  $E^1$ ). The second-year tetraploids slightly exceeded diploids where the pots were biggest and the growth period longest (Series  $B^1$ ). The trend was reversed in first-year plants but the differences were slight. Among the progeny of the rec tetraploid crosses considerable differences appeared. For example, the average diameter at the peak of development was 10.2 in. in the vigorous progeny of  $31 \times 86$  as compared with 7.0 in. in the progeny of  $84 \times 86$ .

### *Leaf Width*

This character varied from plant to plant and with stage of development but tended to be greatest among the tetraploids. As noted in the parental generation, certain tetraploids had narrow leaves and simulated diploids, but the majority possessed spatulate early leaves (2). Table III shows the average width of the distal portions of 10 leaves beginning with the widest and longest leaves and including a similar number of smaller but mature leaves formed later. On the whole the leaves were widest in the vigorous second-year plants of Series  $B^1$ . Measured in April, the mature leaves were 16% wider in  $4n$  than in  $2n$  plants. Since the widest leaves persisted longer in the tetraploids, their average leaf width was maintained through June whereas the width decreased in the diploids (Table III). The leaves of first-year plants (Series  $D^1$  and  $E^1$ ) were narrower, especially among the diploids, and the differences between the  $2n$  and  $4n$  plants were more pronounced. Under similar conditions of growth the progeny of colchicined diploids (Series  $D^1$ ) had slightly wider leaves than the progeny of untreated field diploids (Series

TABLE III

AVERAGE WIDTH IN INCHES OF THE DISTAL PORTION OF MATURE LEAVES IN THE FULLY EXPANDED ROSETTE

Series	Date	2n	4n
<i>B</i> <sup>1</sup>	April	0.87	1.01
	June	0.76	1.02
<i>D</i> <sup>1</sup> All plants	July	0.65 ± .06	0.93 ± .10*
Cross 21 × 296	July	—	0.92 ± .11
296 × 21	July	—	0.85 ± .05
354 × 296	July	—	1.02 ± .10
296 × 354	July	—	1.05 ± .10
71 × 17	July	—	1.05 ± .10
71 × 86	July	—	0.99 ± .16
86 × 71	July	—	1.03 ± .13
31 × 21	July	—	0.82 ± .09
21 × 31	July	—	0.75 ± .09
619 × 629	July	—	0.78 ± .12
<i>E</i> <sup>1</sup>	July	0.59 ± .12	0.83 ± .13

\* Mean and standard deviation.

*E*<sup>1</sup>). The first selection of parental colchicined diploids as possible tetraploids was based upon their thicker, wider leaves (2), and this characteristic was maintained in the *F*<sub>1</sub> plants.

The tetraploids of Series *D*<sup>1</sup> provided an even clearer demonstration of an inheritance mechanism. Here the *F*<sub>1</sub> sister plants from controlled crosses were usually remarkably similar in their leaf characteristics while differing widely from the progeny of other crosses (Table III). The sharp delimitation of the various *F*<sub>1</sub> families as viewed in the garden plot was most striking. For example, the progeny of 31 × 21 and the reciprocal of 21 × 31 had deeply cut leaves averaging 0.82 and 0.75 in. wide, whereas the plants of 71 × 86 and its reciprocal possessed more or less entire leaves 0.99 and 1.03 in. wide.

No measurements were made of total leaf area but it was evident that the greater width of tetraploid leaves did not compensate for their markedly smaller numbers. Estimates from the available data indicated that the ratio of 4n to 2n leaf area was only about 1 : 1.6 in the early July of the first year (Series *E*<sup>1</sup>). Since the tetraploids matured later than the diploids and the largest of the former did not complete growth until some time after the diploids, the differences in leaf area narrowed as the season progressed. In April and May of the second year (Series *B*<sup>1</sup>) the ratio of 4n to 2n leaf area was 1 : 1.1.

#### Leaf Margin

Some plants produced more or less entire leaves throughout the growing period while in others the late spring and early summer leaves were increasingly dissected. For descriptive purposes the various kinds of leaves were classified into five types slightly differing from the five (*A* to *E*) described by Miss

Kern (6) for *Taraxacum palustre* var. *vulgare* and *T. laevigatum*. These are: Type I, margins entire or nearly so; Type II, sinuses less than one-fourth the leaf width; Type III, sinuses one-fourth to one-half the leaf width; Type IV, sinuses one-half to three-fourths the leaf width, and Type V, sinuses exceeding three-fourths the leaf width, extending almost to the midrib. The distribution of leaf types among the first-year population is shown in Table IV. On the

TABLE IV  
DISTRIBUTION OF LEAF TYPES IN FIRST-YEAR PLANTS

Series	Description	Location	Leaf types of parents	Distribution of $F_1$ plants according to leaf type, %					Number of $F_1$ plants
				I	II	III	IV	V	
$D^1$	2n	In pots	—	0	10	47	43	0	42
	4n All plants	In pots	—	2	26	41	31	0	393
	Cross 71 × 17	In pots	II, II-III	0	76	24	0	0	29
	354 × 296	In pots	II, II-III	5	44	30	21	0	43
	296 × 354	In pots	II-III, II-III	0	38	52	10	0	40
	17 × 296								
	296 × 17								
	71 × 86	In pots	II, II	0	28	65	7	0	43
	86 × 71								
	17 × 31								
	31 × 17	In pots	II-III, IV	5	33	54	8	0	39
	71 × 296								
	296 × 71								
	296 × 21	In pots	II-III, III-IV	0	3	30	67	0	33
	21 × 296								
	21 × 31								
$E^1$	2n	In pots	—	0	6	28	60	6	32
	4n	In pots	—	6	17	31	40	6	35
Field	2n	Field	—	1	5	19	61	14	197
	4n	Field	—	9	19	28	45	4	210

whole the incidence of deeply dissected leaves (Types IV and V) was lower in tetraploids than in diploids. Differences of similar order were observed in both potted (Series  $E^1$ ) and field plants. It is noteworthy that potted and field plants grown from similar seed did not differ significantly in their leaf types, diploids and tetraploids exhibiting a similar margin of difference in both cases. The  $F_1$  progeny of colchicined diploids, selected as possible tetraploids (Series  $D^1$ ) were not quite so deeply cut as the progeny of random untreated diploids (Series  $E^1$ ) and in this respect resembled the parental types.

Of the tetraploids derived from controlled crosses (Series  $D^1$ ) the sister plants in each  $F_1$  family were remarkably alike and resembled their parents when both these had the same type of leaf margin. For example, the progeny of 71 × 17 (Table IV) had shallowly cut leaves mostly of Type II, like the

parents. The parental plants 619 and 629 possessed leaves of Type IV and again the progeny resembled the parents, 79% possessing leaves of Type IV (Table IV). When the parents differed markedly in leaf type the progeny were more variable. Thus the cross  $17 \times 31$ , with parental leaf Types II and IV, produced progeny with 5% Type I, 33% Type II, 54% Type III, and 8% Type IV.

### *Summer Dormancy*

Random second-year diploids and tetraploids showed no marked differences in the rosette during summer dormancy, but wide differences occurred between the differently treated series. The plants of Series  $A^1$  resided in the greenhouse throughout winter and summer and cycles of leaf production and flowering followed each other with little evidence of dormancy in either diploids or tetraploids. But in Series  $C^1$ , the plants of which overwintered outside and remained in the garden plot during the summer, both  $2n$  and  $4n$  rosettes almost completely disappeared in a profound dormancy. Though their behaviour was variable, dormancy was more marked among first-year diploids than first-year tetraploids (Series  $D^1$  and  $E^1$ ). The  $F_1$  progeny of the controlled  $4n$  crosses varied greatly, the rosettes of some disappearing almost entirely while other plants grew on to well above the average size.

### *Stomatal Dimensions*

Measurements were made as for the parental generation (2). Although the range was great, guard cells were on the average 38% longer in tetraploids and the stomata only half as frequent as in diploids (Table V). No outstand-

TABLE V  
LENGTH OF GUARD CELLS AND FREQUENCY OF STOMATA

Series	No. of plants	Range and average length of guard cells ( $\mu$ )		Range and average frequency of stomata (per sq. mm.)	
		$2n$	$4n$	$2n$	$4n$
$A^1$	43	26-32 (28.5)	32-47 (38.5)	93-166 (129)	53-117 (75)
$B^1$	50	26-31 (28.5)	35-45 (40.0)	106-180 (134)	43-90 (59)
$D^1$	112	26-36 (30.0)	33-46 (41.0)	84-201 (133)	54-116 (79)
$E^1$	63	26-35 (30.0)	37-46 (41.0)	108-217 (155)	45-115 (70)

ing differences were observed between the various series or between first-year and second-year plants. The marked reduction in size of the stomata in the diploids of Series  $D^1$  as compared with the colchicined parents, which had predominantly diploid inflorescences but were originally selected as possible tetraploids because of their larger stomata, is doubtless evidence that the parental plants were chimaeras with some  $4n$  tissue in their leaves. Among both  $2n$  and  $4n$  plants there was surprisingly little difference between the depauperate plants of Series  $A^1$  and the vigorous plants of Series  $B^1$  in



stomatal dimensions. An attempt was made to determine whether a correlation existed between cell size, as indicated by size of stomata, and weight of roots, but because of variation among leaves of the same plant the data proved insufficient to be conclusive.

### Inflorescences

#### Head Production

Table VI records productivity of diploids and tetraploids from early spring to Aug. 31, 1945. In each of the series the diploids were much more floriferous than the tetraploids. Considerable interserial differences also appeared. Maximum flowering took place in the second-year plants of the optimal Series  $B^1$ , and production was less profuse in the first-year plants of Series  $D^1$  and  $E^1$ , especially among the tetraploids.

TABLE VI  
TOTAL HEAD PRODUCTION JAN. TO AUG. 31, 1945

Series	$2n$		$4n$	
	No. of plants	Range and av. no. of heads	No. of plants	Range and av. no. of heads
$A^1$	22	0-48 (20)	37	0-33 (11)
$B^1$	18	20-123 (64)	38	1-54 (22)
$C^1$	35	12-111 (48)	68	0-37 (16)
$D^1$	43	6-170 (49)	553	0-54 (12)
$E^1$	32	0-133 (23)	34	0-15 (6)

The progeny of the various  $4n$  crosses differed markedly in head production. For instance, the crosses  $21 \times 31$  and  $31 \times 21$  produced an average of 20 and 24 heads respectively in the  $F_1$  generation, whereas  $296 \times 354$  and  $354 \times 296$  produced only 10 and 12 heads. Although there was much variation, Table VII shows that head productivity of the parental plants in their second

TABLE VII  
HEAD PRODUCTION OF PARENTAL AND  $F_1$  TETRAPLOIDS

Parents (second year)	$F_1$ plants (first year)
More than 30	15.0
20 to 30	11.3
Fewer than 20	10.7

year (when they were crossed) ran parallel with that of the progeny in their first year. Differences were less marked in the progeny, possibly because of the relatively sparse flowering of first-year plants.

*Florets*

The number of florets per head varied from plant to plant and with time of flowering, but was usually greater in tetraploids than diploids. The data in Table VIII were compiled from nearly 700 heads. The number of florets per head was least in the suppressed Series  $A^1$  and greater in the luxuriant Series  $B^1$ . The general seasonal trend was toward higher numbers in the

TABLE VIII  
RANGE AND AVERAGE NUMBER OF FLORETS PER HEAD

Series	June		July	
	$2n$	$4n$	$2n$	$4n$
$A^1$	47- 93 (70)	57-105 (80)	40- 78 (60)	45- 99 (71)
$B^1$	40-109 (83)	52-144 (100)	56- 93 (75)	61-114 (90)
$C^1$	40-106 (75)	—	52- 98 (72)	77-136 (97)
$E^1$	—	—	60-114 (85)	68-144 (100)

early heads and decreasing numbers in the later heads, but there were exceptions. On the whole the greater number of florets per  $4n$  head was far outweighed by the superior number of diploid heads. In the floriferous, second-year plants of Series  $B^1$  the total number of florets per  $4n$  plant was only about 40% of that of diploids. The disparity was even greater in first-year plants (Series  $D^1$  and  $E^1$ ).

The linear dimensions of tetraploid florets averaged 25% greater than those of diploids. Structure was essentially similar but abnormalities were more frequent among tetraploids. For instance, in one plant the anthers were short and barely protruded beyond the cleft in the corolla tube. This individual was a poor pollen parent in controlled crossing. Other plants had florets with short, recurved rays allowing the style and staminal tube to thrust far out. A few plants with contorted, shrivelled florets interspersed through the heads were discovered to have cytological irregularities.

*Pollen*

Normal pollen grains were usually 28 to  $30\mu$  in diameter in diploids and 32 to  $40\mu$  in tetraploids. Grain size was more variable in the latter, some plants having consistently large (or less often relatively small) grains, while in others there were varying proportions of large and small grains, many defective. The pollen mother cells of the more variable plants generally revealed meiotic irregularities such as lagging chromosomes and bridges. A small minority of the tetraploids had consistently small, uniform, apparently normal grains. One plant with stomata above average size for tetraploids produced in one head grains of  $35.5\mu$  average diameter and in another grains of  $31.5\mu$ , a size very little above that of diploids. Dermen (3) states that grain size is a more reliable criterion than size of stomata in the distinction of

diploids from tetraploids. This probably holds true for kok-saghyz, but in extreme cases there may be little difference in size.

Some plants, diploid as well as tetraploid, produced no pollen owing to abortion of the mother cells. Some such plants, especially among the diploids, were notably prolific head producers.

### *Fertility*

Seed productivity tended to be lower in the  $F_1$  tetraploids than in the diploids. Nearly a hundred heads of the optimal Series  $B^1$  plants were bagged on three dates: June 5 to 7, when head production had shortly passed the maximum, June 19, and July 5, at the close of blossoming. The two heads of each cross were rubbed together a similar number of times and at seed collection the numbers of achenes and aborted ovaries were counted. Fertility in the diploids crossed on these dates was 53, 17, and 34% as compared with 22, 8, and 5% for tetraploids. Pollination may have been less effective than that by insects, and seed production correspondingly subnormal, but at least conditions were similar for  $2n$  and  $4n$  plants. Moreover, diploids and tetraploids both originated from random seed, so that the chances of relationship between crossed plants were remote.

About one hundred  $F_1$  tetraploids of known parentage (Series  $D^1$ ) were the subject of recorded crosses. Most sister plants proved intersterile, only 18% of the heads involved in 149 different crosses producing achenes. Fertility was no higher in 68 crosses with the reciprocal. When the  $F_1$  plants had one parent in common, seed was set in 27% of the heads in 83 crosses. In 158 crosses between unrelated plants 54% of the heads produced achenes. Inadequate pollination was not the cause of failure; the heads were always thoroughly rubbed together and if any failed to open for any reason, such as cloudy weather, they were discarded.

A few tetraploids were exceptionally cross-fertile. For instance, one individual was fertile with four out of five sister plants. On the other hand, the cross-pollinated heads of the siblings were in each case completely barren, though at least two were fertile when crossed with unrelated plants. Tests of other heads revealed the fertile individual to be neither self-fertile nor apomictic. Evidently kok-saghyz has a complex incompatibility mechanism with occasional plants possessing a different genotype imparting unusual cross-fertility. Stout (11) has reviewed the literature on the inheritance of incompatibility.

### **Roots**

#### *Root Weight*

No outstanding differences in mean root weight were discovered between tetraploids and diploids. Table IX shows that tetraploids exceeded diploids in some series and were inferior in others. The biggest single root, weighing 79 gm., was tetraploid, but the distribution of size in tetraploid roots was little different from that in diploids. Ten per cent of all tetraploids (both first and second-year plants) possessed roots exceeding 30 gm., as compared with 12% of the diploids.

TABLE IX

RANGE AND MEAN FRESH-ROOT WEIGHT IN GRAMS

Series	No. of plants	2n	4n
A <sup>1</sup> (singly in 6-in. pots)	51	5.0-20.0 ( 8.9)	4.0-16.0 (8.2)
B <sup>1</sup> (singly in 9-in. pots)	31	7.0-31.5 (23.0)	15.0-44.5 (26.0)
(two in 9-in. pots)	23	7.0-25.0 (14.5)	6.0-27.5 (16.5)
C <sup>1</sup> (singly in 8-in. pots)	43	11.5-43.5 (25.0)	6.7-33.0 (16.2)
(two in 9-in. pots)	46	9.3-22.5 (16.6)	6.2-24.5 (12.2)
D <sup>1</sup> (singly in 8-in. pots)	513	7.0-32.5 (18.6)	3.0-56.0 (17.8)
E <sup>1</sup> (singly in 8-in. pots)	65	1.5-46.5 (20.1)	6.0-79.0 (22.5)

The smallest roots, both diploid and tetraploid, occurred in the suppressed Series A<sup>1</sup>. The largest roots were developed by the second-year, single-potted plants of Series B<sup>1</sup>, which began growth early in the greenhouse, became the leafiest and most floriferous, and were less affected by summer dormancy. In this series the tetraploids had slightly larger roots than the diploids. In Series C<sup>1</sup> where growth began later, outside, and summer dormancy was profound, the growth of the more lethargic tetraploids was seriously curtailed and at harvest the 4n roots were definitely smaller than those of the diploids. In the first-year plants (Series D<sup>1</sup> and E<sup>1</sup>) that were started indoors in March, so that the 1945 growing period was longer than for Series C<sup>1</sup>, the roots were of moderate size and without marked difference between tetraploids and diploids. Among the diploids, it is noteworthy that the progeny of colchicined plants selected as possible tetraploids but later proved diploid (Series D<sup>1</sup>) were not superior but rather slightly inferior to the control plants derived from untreated field diploids (Series E<sup>1</sup>).

Root weight in the controlled 4n crosses was variable and the data inconclusive. Results from the majority of the crosses studied are set forth in Table X. In some cases there was close agreement between both sets of F<sub>1</sub> progeny and the parents, as in 71 × 296 and 296 × 71. In other instances the two F<sub>1</sub> populations were similar but differed from the parents, as in the crosses 71 × 86 and 86 × 71. Yet another condition obtained in 17 × 31 and 31 × 17 when the progeny differed both from each other and the parents. When considering these results cognizance should be taken of the fact that the F<sub>1</sub> and parental generations had quite different histories, particularly in the early stages of development, and were grown under dissimilar conditions. Further, many of the parents were chimaeras with 4n rosettes and 2n roots. Hence the acquirement of adequate information requires the extension of selection experiments through the F<sub>2</sub> and later generations. It is noteworthy that in all crosses the sister plants exhibited less deviation from the mean root weight than the randomized control tetraploids in Series E<sup>1</sup>. This probably indicates genetical influence. However, the nature of the mechanism is not apparent from the data at hand.

TABLE X

MEAN FRESH-ROOT WEIGHT OF PARENTAL PLANTS (SECOND YEAR) AND  $F_1$  PLANTS (FIRST YEAR)

Series	Description	Mean root weight of parents (gm.)	Root weight of $F_1$ plants		Number of plants
			Mean	Standard deviation	
$D^1$	$2n$	—	18.6	—	43
	$4n$ All plants	—	17.8	—	470
	Cross 578 $\times$ 562	29.8	20.1	6.5	27
	31 $\times$ 21	26.5	16.7	6.9	26
	21 $\times$ 31	26.5	12.6	4.6	19
	31 $\times$ 17	26.5	21.0	6.3	33
	17 $\times$ 31	26.5	11.4	3.4	8
	31 $\times$ 257	22.0	24.1	11.5	17
	21 $\times$ 107	21.5	17.9	7.5	17
	17 $\times$ 296	21.3	15.3	6.2	10
	296 $\times$ 17	21.3	14.7	3.8	24
	31 $\times$ 86	20.0	24.3	11.8	38
	71 $\times$ 17	19.0	18.3	5.3	28
	296 $\times$ 354	18.3	14.4	5.0	27
	354 $\times$ 296	18.3	17.4	6.3	16
	466 $\times$ 469	17.5	9.9	3.4	10
	469 $\times$ 466	17.5	9.2	—	3
	71 $\times$ 296	16.3	15.0	5.0	17
	296 $\times$ 71	16.3	15.2	6.5	9
	621 $\times$ 619	15.7	15.4	3.8	17
	86 $\times$ 84	14.0	10.7	—	3
	71 $\times$ 86	12.5	20.1	10.5	16
	86 $\times$ 71	12.5	23.5	9.2	28
	70 $\times$ 86	8.5	23.2	8.2	14
$E^1$	$2n$	—	20.1	8.8	32
	$4n$	—	22.5	15.3	33

*Root Habit*

The configuration of the root system of tetraploids tended to differ from that of diploids. This was demonstrated in Series  $D^1$  and  $E^1$  where both  $2n$  and  $4n$  plants were handled in the same manner, and damage to the roots was minimized by planting the germinating seedlings directly in small pots rather than in flats. There was much variation but stout undivided tap roots were more prevalent among the tetraploids. On the average the roots of tetraploids had 2.5 large, more or less equal divisions, the diploids 4.8 main parts.

### Relationships between Root Size and Other Characters

In order to determine whether correlation existed between the various vegetative and reproductive characters, data were assembled on root weight, head production, leafiness, and diameter of the rosette in the first-year diploids and tetraploids of Series *D*<sup>1</sup> and *E*<sup>1</sup>, all of which were grown under similar conditions. The data are presented in Table XI.

TABLE XI

RELATIONSHIPS BETWEEN FRESH-ROOT WEIGHT, HEAD PRODUCTION, LEAFINESS, AND SIZE OF ROSETTE IN FIRST-YEAR DIPLOIDS AND TETRAPLOIDS

Type	Root weight, gm.	No. of plants	Av. no. of heads	Av. number of leaves			Av. max. diam of rosette, in.
				July 10	At max.	Aug. 15	
2 <i>n</i>	To 9.9	18	16.3	26.2	26.5	22.9	7.9
	10-19.9	29	36.0	41.8	48.2	43.0	9.3
	20-29.9	29	37.9	44.0	62.5	59.9	9.5
	Over 30	7	53.3	46.7	70.0	70.0	10.4
4 <i>n</i>	To 9.9	30	11.5	18.4	19.8	15.2	7.9
	10-19.9	141	12.8	20.3	26.1	25.1	8.8
	20-29.9	96	10.8	20.8	31.1	30.8	9.4
	30-39.9	37	9.9	23.3	45.0	44.0	10.0
	Over 40	10	10.1	26.7	54.1	54.0	11.6

Absence of flowering during the first year has been reported in the Russian literature, reviewed by Krotkov (7), as associated with large root size in diploid kok-saghyz, and it was suggested that selection should be made on that premise. The data in Table XI clearly do not support such a conclusion. Among the diploids head production during the first year was extremely variable, as illustrated by the range, 7 to 170 heads in the largest specimens (with roots exceeding 30 gm.), but, on the whole, plants with the biggest roots were the most floriferous, and conversely those with the smallest roots tended to have fewest heads. In the tetraploids, where the number of plants was greater, a different trend appeared. Here flowering was much reduced, with the maximum in plants possessing roots somewhat below average size (10 to 19.9 gm.). However, because of the great variability among individuals it was clear that neither high nor low productivity of heads, in either diploids or tetraploids, could be considered prognostic of ultimate root weight. There were indications that the smaller plants tended to flower earlier than those that finally became largest, but dates of the initiation and duration of flowering were not recorded.

Leaf shape was another character regarded by one Russian investigator as useful for selecting kok-saghyz, but has been discounted by others (Krotkov (7)). Records were not kept of the leaf shape of all the first-year tetraploids (Series *D*<sup>1</sup> and *E*<sup>1</sup>) but the available data are given in Table XII. At least in

TABLE XII  
FRESH-ROOT WEIGHT AND LEAF TYPE IN FIRST-YEAR TETRAPLOIDS

Root weight (gm.)	Distribution of plants according to leaf type				
	I	II	III	IV	V
To 9.9	1	13	22 (45%)	13	0
10-19.9	3	61	90 (39%)	78	1
20-29.9	0	23	40 (44%)	28	0
Over 30	0	7	11 (41%)	6	3

these plants there was no obvious relationship between leaf type and root weight.

Nichiporovich and Ivanitzkaja (9) reported a correlation between numbers of leaves produced during the period of most active growth and ultimate root size in diploid kok-saghyz. A generally similar relationship was observed in the first-year plants, both diploid and tetraploid, of Series *D*<sup>1</sup> and *E*<sup>1</sup> (Table XI). Although highly variable, plants that were leafiest in July tended to produce the biggest rosettes and roots. However, it is clear from the data that the larger size of these plants was due not so much to more rapid early growth as to lengthening of the growing period. The rosettes of plants that produced the smallest roots, weighing up to 9.9 gm., in most cases had achieved full expansion in early July and by Aug. 15 had undergone considerable diminution. While the rosettes of the small plants were dying back, those of the large plants continued to expand, and in the case of the biggest this expansion was not completed until mid-August (Table XI). The relative state of leafiness on Aug. 15 as contrasted to July 10 was in the order of -13, +13, +36, and +50% in the four categories of diploids with progressively larger roots, and -16, +24, +48, +89, and +102% in tetraploids. The greater increase during the latter part of the growing season in tetraploids is further illustration of their physiological tardiness. They grew more slowly than diploids but duration of the first cycle of growth extended deeper into the summer.

Of the various characters studied, diameter and leafiness of the rosette appeared to be the best indicators of the ultimate root size. However, since the average differences during the early stages tended to be slight, especially in the tetraploids, and the variation among individuals was great, this character proved of limited value when put to actual test. Many of the plants selected for crossing because of their promising appearance at time of flowering were finally found to have roots of average size. Conversely the potentialities of other plants were unrecognized in early summer because much of their ultimate bulk was due to prolonged rather than rapid growth. The attempts at selection for root size during early summer were generally discouraging, and it seemed clear that a longer program was required with crossing deferred until after the completion of summer growth.

## Discussion

In the present paper comparative data are presented on three categories of kok-saghyz plants grown in pots. The types are random diploids,  $F_1$  diploids derived from colchicined plants selected as possible tetraploids on their leaf characters but later determined as predominantly diploid, and the  $F_1$  generation of colchicine-induced tetraploids.

The progeny of selected colchicined diploids differed from the random derivatives of untreated field diploids in possessing leaves that were slightly wider, less deeply dissected, and somewhat less numerous. In these leaf characters they resembled the parental plants. Size of stomata, however, was much reduced and approximated that in random diploids. Rosettes were no larger, and root size was slightly below that of random diploids, though the inferiority was so minor as probably to be without significance.

The  $F_1$  tetraploids exhibited the morphological modifications described repeatedly in the extensive literature of polyploidy. All parts of the plant body were more robust than in diploids. The leaves were usually wider and less dissected, but the increase in width was counteracted by the marked reduction in number, and only at maturity did leaf area of the tetraploids approximate that of the diploids. Similarly  $4n$  inflorescences were larger and contained more florets, but the heads were so reduced in number that even second-year tetraploids produced only 40% as many florets as diploids. Since fertility was also definitely lower, achene production was but a small fraction of that in  $2n$  plants.

Sibling tetraploids were notably intersterile, fewer than one-fifth of the crosses between sister plants being successful. With decrease in genetic relationship cross-fertility increased. As a rule the induction of tetraploidy brought no release from the operation of the incompatibility mechanism possessed by kok-saghyz. Reports on other genera indicate varied effects of polyploidy on incompatibility. Stout (12) described the origin of self-fertile  $4n$  petunias from self-sterile  $2n$  plants. Lewis and Modlibowska (8) found a definite increase in self-fertility in tetraploids derived from an only slightly self-fertile diploid clone of the cultivated pear. On the other hand, Hecht (5) reported persistence of self-sterility in tetraploid *Oenothera rhombipetala*. Evidently kok-saghyz belongs in that category of plants whose incompatibility mechanisms persist despite polyploidy.

No outstanding differences in mean fresh-root weight were discovered between tetraploids and diploids, the former showing minor superiority in some series and slight inferiority in others. The roots of the tetraploids were usually more robust, but the increase in thickness was offset by reduction in number of major divisions so that the average weight was not greatly different.

In their growth behaviour tetraploids were more torpid than diploids. Individuals were highly variable, but generally the rosettes of tetraploids underwent slightly slower increase in diameter, leaf turnover was greatly reduced, flowering began slightly later, head production was much lower,



fertility greatly reduced, and summer dormancy tended to be postponed. This tardiness seems characteristic of tetraploids generally. Causal factors have not been investigated in many cases, but Noggle (10) in his report on rye described the tetraploids as being physiologically younger than diploids of similar chronological age. Avery and Pottorf (1) and Gustafson (4) found the concentration of growth hormones to be lower in tetraploids.

In the various  $F_1$  generations of tetraploids produced by controlled crossing, sister plants exhibited less deviation from the mean than randomized plants in size, shape, and number of leaves, production of heads, and size of roots. Of these characters, leaf shape provided the clearest demonstration of an inheritance mechanism. Head production also appeared to be inherited though not so precisely as leaf shape. The data on root weight were less conclusive and selection experiments need to be continued to the  $F_2$  and succeeding generations.

It should be emphasized that the descriptions in this paper refer to potted plants. There were indications both from these and from plants grown in the field that when the more lethargic tetraploids were given opportunity to achieve full growth, they sometimes surpassed diploids. Warmke (13) reported the roots of field-grown tetraploids to be twice as heavy as those of diploids, but the number of plants was small. In plots at Toronto the tetraploids exceeded diploids by a small but definite margin. In general the tetraploids gave the impression of having possibilities of surpassing diploids, but seemed held back by undetermined deficiencies in their growth mechanism. Whether the growth characteristics of the outstandingly large tetraploids are heritable remains to be discovered.

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## STUDIES WITH *BACILLUS POLYMYXA*

### IV. NITROGEN REQUIREMENTS IN RELATION TO 2,3-BUTANEDIOL PRODUCTION FROM STARCH<sup>1</sup>

BY H. KATZNELSON<sup>2</sup>

#### Abstract

Yeast extract, casein hydrolysate, and a mixture of 13 to 20 amino acids were found to be superior to simpler substances such as ammonium sulphate, urea, potassium nitrate, or asparagine as sources of nitrogen for *Bacillus polymyxa* in relation to production of 2,3-butanediol from starch. The complex sources of nitrogen were more or less interchangeable for most, but not all, strains of this organism with regard to effectiveness for diol production, but the addition of yeast extract to either casein hydrolysate or amino acids resulted in a slightly higher yield of diol and an increase in the diol/ethanol ratio from 2 to 2.6 or higher.

The requirements of different strains for specific amino acids varied somewhat, but the need for isoleucine and asparagine was common to the four strains studied. A fairly good fermentation was obtained with the most efficient of these strains in a medium containing isoleucine, tyrosine, glycine, methionine, and asparagine after three days' incubation. The fermentation went to completion after five days with certain concentrations of these five acids, an effect that was achieved in three days by the addition to these of eight other acids but not by the addition of ammonium sulphate. Suppression of diol production by omission of certain of these amino acids was marked at three days but was largely overcome at five. Cystine (in concentrations above 0.0125%) was inhibitory to the four strains studied and phenylalanine (0.02%) to one, after three but not after five days.

#### Introduction

In the preceding paper of this series (8) it was shown that *Bacillus polymyxa* could grow in a medium containing casein hydrolysate as the nitrogen source and that this complex could be replaced by a mixture of 18 amino acids, urea, or ammonium sulphate. Yeast extract was found to be superior to all these substances and has also been shown to exert a favourable effect on production of 2,3-butanediol from wheat (7, 9). Subsequent to the publication of the above work (8) growth studies were continued with other strains of *B. polymyxa*; it soon became apparent that for most of these, casein hydrolysate, a mixture of amino acids (slightly different from that used in the earlier investigation), or yeast extract was far superior to simpler compounds such as urea or ammonium sulphate. Growth with amino acids was usually equal, and often superior, to that with casein hydrolysate, but yeast extract was always the most effective substance. However, attempts to

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replace the latter with 15 growth-promoting substances (vitamin B complex, purine, and pyrimidine bases) in a medium containing casein hydrolysate or amino acids as a source of nitrogen were unsuccessful. Further studies were made to determine which of the amino acids used were essential for growth of this organism. The omission of leucine or isoleucine from a mixture of 18 acids retarded growth appreciably at 48 hr. but this effect was not evident after 96 hr.; none of the remaining acids appeared to exert any specific effect.

Similar work was carried out on the influence of various nitrogenous substances on production of 2,3-butanediol rather than on growth. Starch was used as the carbon source instead of glucose since the latter was found to be toxic to *B. polymyxa* in the concentrations required to give appreciable yields of the diol. The proposed utilization of starch as the substrate for production of butanediol (4, 9, 17), owing to difficulties encountered in the use of whole wheat, heightened the interest in this investigation, the results of which are reported in this paper.

### Experimental

A basal medium containing, per litre, 0.3  $\gamma$  biotin and 5 ml. each of inorganic solutions *A* and *B* of Snell and Strong (15) was prepared in double strength and dispensed in 50 ml. amounts in 500 ml. Erlenmeyer flasks. The ingredients to be tested were then added and the volume of the solution brought to 100 ml., after which the reaction was adjusted to pH 6.8 and 4 gm. of Stein-Hall wheat starch added. The medium was sterilized for 20 min. at 15 lb. pressure and after cooling received 1 gm. calcium carbonate (sterilized separately in tubes). Inoculum was prepared by centrifuging a 24-hr. culture (in casein-hydrolysate-biotin-glucose medium) and resuspending the cells in 10 ml. sterile distilled water, 1 ml. being used per flask. After an incubation period of 72 or 120 hr. at 28° C. the contents of the flasks were filtered and the filtrates analysed for 2,3-butanediol (9). Since changes in diol production were usually accompanied by similar changes in production of ethyl alcohol (diol/ethanol ratio being about 2), analyses for the diol alone were usually made. Where the results suggested a possible variation of this ratio, ethanol was also determined. The data given in the tables are representative of results obtained in frequently repeated experiments. All glassware was carefully cleaned (with acid dichromate) and rinsed.

A 4% starch suspension was found to be the most convenient to work with as a greater concentration cooled to a gelatinous mass that was difficult to manipulate. Malting would have permitted use of higher concentrations of starch but this was of course impossible in these studies in view of the necessity of avoiding introduction of unknown organic substances (other than those present in the starch itself). According to the calculations of Ledingham *et al.* (9), a theoretical yield of diol and ethanol (designated "total products") of 2.2% may be expected from 4% starch, although they state that this may never be obtained even under optimum conditions. However, 85 to 95% of this theoretical yield was usually obtained with the most efficient strain in the collection (No. 47).

## Experimental Results

### *Influence of Different Sources of Nitrogen*

A preliminary survey was made in which various nitrogenous compounds were tested in different concentrations. The results summarized in Table I indicate the following order of effectiveness for butanediol production: vitamin-free casein hydrolysate > yeast extract > mixtures of amino acids > ammonium sulphate > asparagine > urea, and potassium nitrate.

TABLE I

INFLUENCE OF VARIOUS NITROGEN COMPOUNDS ON STARCH FERMENTATION  
BY *Bacillus polymyxa* (STRAIN 47)

Nitrogen source (gm./flask)		Butanediol, %	Nitrogen source (gm./flask)		Butanediol, %
Ammonium sulphate	0.16	0.53	Casein hydrolysate	0.2	1.11
	0.32	0.44		0.4	1.32
	0.48	0.52		0.6	1.39
Urea	0.10	0.01	Amino acids*	0.2	0.95
	0.20	0.01		0.4	1.07
	0.30	0.01		0.6	1.11
Potassium nitrate	0.23	0.09	Yeast extract	0.2	—
	0.46	0.06		0.4	1.17
	0.69	0.08		0.6	1.32
Asparagine	0.3	0.16			
	0.6	0.14			
	0.9	0.11			

\* See Appendix for concentrations used.

This order is somewhat different from that obtained in growth studies, but shows clearly that complex sources of nitrogen are far superior to simple substances. The effectiveness of casein hydrolysate, even that prepared by the same manufacturing concern, was found to vary with the lot used. One lot was definitely inferior to yeast extract for all strains for both growth and diol production; a second (that used in this work), though inferior for growth, was equal to yeast extract for most strains for diol production.

Various combinations of these compounds were then tested as shown in Table II. Evidently the most effective combination is yeast extract with either casein hydrolysate or amino acids, giving a yield of diol of 1.45%, which with 0.55% ethyl alcohol results in a yield of 2.0% total products or 91% of theoretical. Whether the influence of the yeast extract is due to nitrogenous constituents, growth factors, or both is not apparent. Certain strains gave higher yields of diol with yeast extract than with casein hydrolysate, but all produced most efficiently when these two complexes were combined. It will also be noted that yeast extract increased the diol/ethanol ratio to 2.6 to 2.8 as compared with 1.8 to 2.2 for other compounds or combinations of these.

TABLE II

INFLUENCE OF COMBINATIONS OF NITROGENOUS SUBSTANCES ON PRODUCTION OF 2,3-BUTANEDIOL FROM STARCH

Nitrogen source	Butanediol, %	Ethanol, %	Total products, %	Diol
				Ethanol
Ammonium sulphate (0.32 gm./flask)	0.45	0.25	0.70	1.8
Casein hydrolysate (0.4 gm./flask)	1.28	0.64	1.92	2.0
Amino acids (0.4 gm./flask)	1.21	0.58	1.79	2.1
Yeast extract (0.4 gm./flask)	1.24	0.46	1.70	2.7
Ammonium sulphate + casein hydrolysate	1.27	0.57	1.84	2.2
Ammonium sulphate + amino acids	1.06	0.50	1.56	2.1
Ammonium sulphate + yeast extract	1.23	0.44	1.67	2.8
Casein hydrolysate + amino acids	1.27	0.59	1.86	2.1
Casein hydrolysate + yeast extract	1.45	0.55	2.00	2.6
Amino acids + yeast extract	1.46	0.54	2.00	2.7

### Amino Acid Requirements

The effect of omitting amino acids singly from a mixture of 20 (listed in the appendix) on the fermentation of starch by *B. polymyxa* (strain 47) is clearly shown in Table III. The omission of glycine, isoleucine, methionine,

TABLE III

AMINO ACID REQUIREMENT FOR THE PRODUCTION OF 2,3-BUTANEDIOL BY *Bacillus polymyxa*\* (STRAIN 47)

Amino acid omitted	Butanediol, %	Amino acid omitted	Butanediol, %
None	1.13	Tryptophane	1.16
Glycine	0.74	Cystine	1.18
Alanine	1.12	Lysine	1.14
Valine	1.12	Histidine	1.13
Leucine	1.13	Arginine	1.11
Aspartic acid	1.25	Proline	1.13
Glutamic acid	1.25	Isoleucine	0.75
Serine	1.23	Methionine	0.84
Phenylalanine	1.10	Asparagine	0.83
Tyrosine	0.98	Cysteine	1.25
		Threonine	1.17

\* Cystine added at rate of 10 mgm./100 ml. medium.

asparagine (and tyrosine to a slight extent) resulted in depression of diol production. This was confirmed in repeated experiments one of which, along with ethanol analyses, is presented in Table IV. In this and subsequent experiments, cystine, cysteine, and threonine were omitted, and a slightly higher yield was obtained, which was comparable with that given by casein hydrolysate or yeast extract. As will be shown later, this effect was attribut-

TABLE IV

INFLUENCE OF CERTAIN AMINO ACIDS ON PRODUCTION OF 2,3-BUTANEDIOL AND ETHANOL FROM STARCH

Amino acids used	Butanediol, %	Ethanol, %	Total products, %	Diol
				Ethanol
1. All except cystine, cysteine, and threonine	1.31	0.62	1.93	2.10
2. 1-isoleucine	0.87	0.42	1.29	2.10
3. 1-tyrosine	1.19	0.60	1.79	1.97
4. 1-glycine	0.95	0.49	1.44	1.92
5. 1-methionine	0.79	0.39	1.18	2.01
6. 1-asparagine	1.01	0.48	1.49	2.10
7. Isoleucine, tyrosine, glycine, methionine, and asparagine*	1.14	0.55	1.69	2.06
8. 7 doubled	1.20	0.54	1.74	2.22
9. 7 + leucine, tryptophane, arginine, proline, alanine, valine, histidine, lysine	1.32	0.63	1.95	2.10

\* In 7, double concentration normally used.

able to cystine, which appeared to be inhibitory in certain concentrations. It will be noted from Table IV that five amino acids together (with concentrations doubled or quadrupled to maintain the nitrogen level) gave good yields of diol (1.14 to 1.2%) and that the addition of eight other acids, none of which appeared to be necessary (Table III), resulted in a yield of butanediol (and total products) equal to that obtained with 17 acids. The yield of ethyl alcohol followed closely that of the diol, a ratio of 2 to 2.1 being obtained in most cases.

According to the work of Stokes and Larsen (16) "*Acetobacter suboxydans* can utilize (NH<sub>4</sub>)-nitrogen for growth provided it is supplied with the 6 amino acids which it is unable to synthesize or which are formed too slowly for normal development." An attempt was therefore made to determine whether *B. polymyxa* would ferment starch rapidly in the presence of ammonium sulphate provided the five amino acids mentioned above were also present in the medium. It is clear (Table V) that ammonium sulphate is inferior to the five amino acids both at three and five days even though it supplied about four times as much nitrogen as these substances. These two sources of nitrogen combined resulted in a yield of butanediol that was equivalent to the sum of the yields of both components (Treatments 1 and 2) and that was no better at three days than that obtained by doubling the concentration of the five acids. Maximum yields were obtained in three days only on the addition of eight other acids. However, the fermentation will go to completion with the five amino acids alone (with concentrations doubled) if the incubation period is extended to five days (Table V, Treatment 4). This applies as well to conditions in which these acids are individually omitted from a mixture. As the data in Table VI bear out, the marked retardation



TABLE V

COMPARISON OF AMINO ACID MEDIA WITH AND WITHOUT AMMONIUM SULPHATE IN RELATION TO STARCH FERMENTATION

Addenda to basal medium	Incubation (days)	
	3	5
	Diol, %	
1. Ammonium sulphate (0.3%)	0.45	0.86
2. Isoleucine, tyrosine, glycine, methionine, asparagine	0.78	1.16
3. 2 + 1	1.13	1.20
4. 2 (double amounts)	1.19	1.33
5. 4 + 1	1.17	1.31
6. 13 amino acids*	1.34	1.36
7. 6 + 1	1.32	1.33

\* Five above (in 2) + leucine, tryptophane, arginine, proline, alanine, valine, histidine, lysine.

TABLE VI

EFFECT OF OMITTING CERTAIN AMINO ACIDS ON STARCH FERMENTATION AT THREE AND FIVE DAYS

Acids used	Incubation (days)	
	3	5
	Diol, %	
1. All except cystine, cysteine, threonine	1.43	1.39
2. 1— isoleucine	0.80	1.29
3. 1— methionine	0.72	1.10
4. 1— glycine	0.86	1.31
5. 13 acids*	1.34	1.39

\* See Table V.

of fermentation after three days, resulting from the omission of isoleucine, glycine, or methionine from the amino acid mixture is largely overcome (except possibly with methionine) after five days' incubation, a fact that was noted earlier in growth studies.

The response of different strains to these amino acids varies, as will be seen in Table VII. Omitting isoleucine and asparagine reduces the yield of diol by all three strains; glycine is required by strain 39, and methionine to some extent; tryptophane and arginine are required by strain 252. Thirteen acids are equal to 17 for strain 39, better for strain 252, and not as effective for strain 82. This last observation was again brought out in a comparison of different sources of nitrogen as shown in Table VIII. The four treatments seem of equal value for strains 39 and 47. Again, with strain 82, 13 acids are

TABLE VII

AMINO ACID REQUIREMENTS OF THREE STRAINS OF *Bacillus polymyxa*  
FOR PRODUCTION OF 2,3-BUTANEDIOL

Amino acids used	Strain		
	39	82	252
	Diol, %		
1. All except cystine, cysteine, and threonine	0.93	0.95	0.76
2. 1-isoleucine	0.78	0.73	0.52
3. 1-tyrosine	0.86	0.85	0.73
4. 1-glycine	0.57	0.90	0.81
5. 1-methionine	0.78	0.86	0.74
6. 1-asparagine	0.76	0.61	0.52
7. 1-leucine	0.91	0.95	0.72
8. 1-tryptophane	0.92	0.98	0.55
9. 1-arginine	0.93	0.94	0.62
10. 13 acids*	0.98	0.80	0.95

\* See Table V.

TABLE VIII

INFLUENCE OF SOURCE OF NITROGEN ON PRODUCTION OF 2,3-BUTANEDIOL FROM  
STARCH BY DIFFERENT STRAINS OF *Bacillus polymyxa*

Nitrogen source	Strain			
	39	47	82	252
	Diol, %			
Casein hydrolysate	1.04	1.37	0.94	1.01
17 Amino acids	1.09	1.43	0.95	0.67
13 Amino acids	1.04	1.39	0.77	0.99
Yeast extract	1.09	1.36	0.96	1.13

inferior to 17 and with strain 252, superior. Experiments were then carried out to determine the factor responsible for this phenomenon and phenylalanine repeatedly implicated as stimulatory for strain 82 and inhibitory for strain 252. This effect was not demonstrable after five days' incubation.

It was pointed out above that the omission of cystine from the amino acid mixture gave increased yields of 2,3-butanediol. To settle this point an experiment was carried out in which different amounts of cystine were weighed directly into flasks containing casein hydrolysate as a source of nitrogen. The results (after three days' incubation) show (Table IX) that 25 mgm. cystine inhibited all four strains and that 12.5 mgm. was somewhat inhibitory also, especially for strain 82. The four strains tolerated at least 6 mgm. cystine per 100 ml. of medium. This repressive action was not permanent and could not be demonstrated after five days.

TABLE IX

INFLUENCE OF L-CYSTINE ON FERMENTATION BY FOUR STRAINS OF *Bacillus polymyxa*  
GROWING IN A CASEIN-HYDROLYSATE-STARCH MEDIUM

L-Cystine (mgm./100 ml. medium)	Strain			
	39	47	82	252
	Diol, %			
0	0.97	1.21	0.71	0.88
6	0.79	1.22	0.61	0.85
12.5	0.81	1.12	0.44	0.81
25.0	0.64	0.89	0.47	0.61

### Discussion

Ledingham *et al.* (9) suggested that it might be preferable to remove the non-fermentable fractions of wheat for the development of an economical 2,3-butanediol fermentation. Ward *et al.* (17) also suggest that the recovery of butanediol would be simpler if starch rather than whole grain were used as the fermentation substrate. The former workers pointed out too that before this could be done "a more comprehensive knowledge of the nutritional requirements of *Aerobacillus polymyxa* is essential." Pure starch with inorganic nutrients was not fermented satisfactorily. Moderately good fermentations resulted from the incorporation of 7.5 to 10% (on weight of mash) wheat germ, shorts, or bran in a pure starch medium but these were impractical quantities. Whole wheat mash itself was found to be improved by yeast extract but not by gluten. These workers concluded that the nitrogen requirements of this organism could be met largely by soluble nitrogenous substances. In further work along these lines Fratkan and Adams (4) also demonstrated the stimulatory effect of bran and shorts in an 8% starch medium on 2,3-butanediol production. Of various other supplements tested, malt sprouts proved to be the most effective, being superior to yeast extract as well.

The use of these complex sources of nitrogen gives no indication of the specific nitrogen requirements of this organism and in fact very little has been published on this aspect of its nutrition. Perhaps the most comprehensive study along this line is that of den Dooren de Jong (1) in which many substances, including amino acids, amines, amides, and urea derivatives, were added singly to different inorganic media with and without glucose. Depending on the conditions, many of these substances could or could not be utilized. It is interesting to note that under conditions favouring the utilization of most of the amino acids no growth was obtained with cystine, which was shown in the present study to be actually inhibitory in certain concentrations. Grossowicz (6) found cystine to be inhibitory for *Neisseria intracellularis*. Snell and Guirard (14) showed that glycine, and to a lesser extent serine and

threonine inhibited their "*Streptococcus lactis* R" culture although this inhibition could be overcome by addition of pyridoxine or alanine to the medium. Niven and Sherman (12) obtained complete inhibition of five enterococci by large amounts of glycine (20 mgm./10 ml.) a phenomenon that was also observed with *Streptococcus lactis* by Niven (11). Greene (5) noted a significant reduction in the growth of *Leptospira canicola* by 10 amino acids including phenylalanine when the amounts used were 0.025% or greater. Fox *et al.* (3) and Fling and Fox (2) demonstrated inhibition of growth and acid production of *Lactobacillus arabinosus* 17-5 by *d*-leucine and *d*-valine but not by their isomers *l*-leucine and *l*-valine. It appears, therefore, that certain amino acids may be inhibitory although the reasons for this effect are obscure as yet.

The results presented in this paper confirm the conclusions of Ledingham *et al.* (9) that soluble nitrogenous materials fulfil the nitrogen requirements of *B. polymyxa*. For most strains, yeast extract, casein hydrolysate, or a mixture of amino acids are interchangeable; however, the first of these exerts an additional stimulating effect when combined with either of the other nitrogen sources, and also possesses the capacity of increasing the diol/ethanol ratio from 2 to 2.6 or more.

The amino acid requirements of strains of *B. polymyxa* vary somewhat, although the need for isoleucine and asparagine seems to be common to the four strains studied. Similar variation of requirements of strains for amino acids has been noted for *Streptococcus salivarius* (13), for *Streptococcus lactis* (11), and for enterococci (12). Furthermore, as Niven (11) and Stokes and Larsen (16) have pointed out, certain amino acids are undoubtedly more important than others; nevertheless satisfactory growth does not occur unless a number of acids, whose individual omission from a mixture does not affect growth, are included in the medium. Whether these acids function merely to raise the nitrogen level of the medium to a certain optimum concentration or whether they function synergistically with other constituents of the medium or both is not understood at present. They may actually be required by the organism, a fact that is not apparent when they are omitted singly from a mixture of 20 acids because of possible transamination reactions (10) or utilization by the organism of closely related amino acids in the mixture. *B. polymyxa* appears to have the capacity to compensate for omissions of important acids by synthesis or utilization of closely related compounds provided it is given sufficient time to do so. The protein still remaining in the starch (0.375% = 15 mgm./100 ml. medium) contributes very little to the nitrogen requirements of the organism.

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## APPENDIX

## AMINO ACIDS USED (MILLIGRAMS PER LITRE)

Glycine	80	<i>l</i> -Cystine	250
<i>dl</i> -Alanine	80	<i>dl</i> -Lysine dihydrochloride	400
<i>dl</i> -Valine	320	<i>l</i> -Histidine hydrochloride	160
<i>l</i> -Leucine	100	<i>d</i> -Arginine hydrochloride	160
<i>l</i> -Aspartic acid	100	<i>l</i> -Proline	200
<i>d</i> -Glutamic acid	500	<i>dl</i> -Isoleucine	200
<i>dl</i> -Serine	80	<i>dl</i> -Methionine	160
<i>dl</i> - $\beta$ -Phenylalanine	200	<i>l</i> -Asparagine	500
<i>l</i> -Tyrosine	140	Cysteine hydrochloride	200
<i>l</i> -Tryptophane	200	<i>dl</i> -Threonine	100

## ENTYLOMA FUSCUM AND RELATED SMUTS ATTACKING PAPAVERACEAE<sup>1</sup>

BY D. B. O. SAVILE<sup>2</sup>

### Abstract

A detailed morphological study has led to the conclusion that *Entyloma Glaucii* should be included in *E. fuscum*. The symptoms of the smut on *Papaver Rhoeas* and *P. somniferum* are described. Study of limited material suggests that the fungus on *Eschscholzia* and *Dendromecon* in California should be retained as *E. Eschscholtziae*. The production of conidia on these hosts is reported for the first time. *Entyloma fuscum* from *Papaver Rhoeas* has been shown to attack *P. atlanticum*, *P. dubium*, *P. glaucum*, *P. monanthum*, *P. orientale*, *P. setigerum*, and *P. somniferum*.

### *Entyloma fuscum* Schroeter

*Entyloma fuscum* Schroeter, causal fungus of the leaf smut of poppies, has been reported from Europe on *Papaver Argemone*, *P. dubium*, *P. nudicaule*, *P. Rhoeas*, and *P. somniferum*. In addition, the fungus on *Glaucium flavum*, segregated by Dangeard as *E. Glaucii*, and that on *G. corniculatum*, segregated by Ciferri as *E. Fragosi*, are regarded by Liro (4) as belonging to this species. The important European literature references for this smut are listed by Liro and will not be repeated here.

Material recently collected by the writer has led to a study of *Entyloma fuscum*. Inoculation tests have somewhat increased the known host range; and detailed measurements have been made in order to permit a more adequate comparison of the smut on poppy with those on other Papaveraceae. The writer is indebted to Dr. G. L. Zundel, Pennsylvania State College, for specimens of the smut on *Dendromecon* and *Eschscholzia*, and to Prof. H. S. Jackson for the opportunity to examine material in his herbarium and that of the University of Toronto.

*Entyloma fuscum* has been reported several times in North America, but it has apparently never become firmly established, perhaps because of the scarcity of wild hosts. Its sporadic occurrence suggests that it is carried with the seed in fragments of leaves or capsules. Clinton (2) mentions its occurrence in Maine and New Brunswick, and Gilman and Archer (3) record it from Iowa, all on *Papaver* sp. Zundel (9) records it from Bermuda on *P. Rhoeas* and *P. somniferum*. In addition, Prof. H. S. Jackson has a specimen in his herbarium from Sherwood, N.Y., on *P. somniferum*. It may be noted that Seym. and Earle Econ. Fungi C 19 and Reliquiae Farlowianae 693, collected at Kittery Point, Me., in 1889 and 1891, respectively, were issued as on *Papaver* sp., but that both are almost certainly on *P. somniferum*. The writer is obliged to Dr. D. H. Linder for the information that the New

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Brunswick record stems from a specimen in the Farlow Herbarium collected at Campobello in 1898 by Farlow, and that the host appears to be *P. somniferum*.

In 1944 this smut was found on *Papaver Rhoeas* in three gardens at Westboro, near Ottawa, Ont., as has already been recorded (5). Further search in 1945 revealed infection of *P. somniferum* in two gardens and of the more commonly grown *P. Rhoeas* in about 10.

Slightly infected *P. orientale* was found in 1944 adjacent to infected plants of *P. Rhoeas*. No conidia were formed on *P. orientale* and the teliospores were somewhat less abundant than in lesions on *P. Rhoeas*. A section through a typical lesion on this host is shown in Fig. 1. It may be mentioned that this photograph, as well as those in Figs. 2, 3, and 5, are from mounts prepared by a rapid sectioning technique devised by the writer (6) in the course of studies of the genus *Entyloma*. By this method sections adequate for such purposes can be secured at a great saving of time. Although there is little difference in the range of spore sizes, the spores in *P. orientale* (Fig. 3) seem to be, on the average, somewhat smaller than in *P. Rhoeas* (Fig. 2). It is doubtful whether the smut, or at least this strain of it, could persist on isolated plants of oriental poppy.

The large, often coalescing lesions on the leaves of *P. somniferum* may seriously disfigure and weaken the plants. On *P. Rhoeas*, of which many named varieties seem to be almost equally susceptible, the leaf injury is generally less severe; under certain conditions, however, extensive lesions may form on the stem, which is often completely girdled when the plant has produced only one or two flowers. This symptom was conspicuous in the hot, dry weather that was experienced in 1944, but was much less frequent in the cooler and moister summer of 1945. Whether it is actually correlated with high temperature or is induced by frequent sprinkling of the plants under such conditions is uncertain. No conidia have been found on these stem lesions, but teliospores are abundant. The teliospores tend to be longer than in the leaves, having been found up to a maximum of  $30\mu$  in length; they lie immediately beneath the epidermis, and mounts are conveniently made by stripping off the epidermis with the spores adherent to it (Fig. 4).

A considerable collection of seeds of Papaveraceae had been brought together for planting in the Dominion Arboretum and Botanical Garden in 1945, and the writer was able to utilize some of these species for inoculation with the smut from *P. Rhoeas*. Infection was secured on *P. atlanticum*, *P. dubium*, *P. glaucum*, *P. monanthum*, *P. setigerum*, and *P. somniferum*. Of these species all but *P. dubium* and *P. somniferum* appear to be new hosts. No infection occurred on *P. Argemone*, *P. nudicaule*, *Argemone Barclayana*,

FIGS. 1 to 5. *Entyloma fuscum*. 1. Section through leaf of *Papaver orientale* showing teliospore distribution,  $\times 135$ . 2. Spores in leaf of *P. Rhoeas*,  $\times 1180$ . 3. Spores in leaf of *P. orientale*,  $\times 1180$ . 4. Spores in stripping from stem lesion on *P. Rhoeas*,  $\times 1180$ . 5. Cyst in mesophyll cell of *P. somniferum*; two spores are seen above,  $\times 1180$ .







*A. grandiflora*, *Celidonium majus*, *Eschscholzia californica*, *E. sp.*, *Meconopsis Pratti*, and *Roemeria sp.* The lack of infection on *P. Argemone* and *P. nudicaule* in this test, and of *P. nudicaule* near infected poppies in several gardens at Westboro, suggests that specialization exists in this smut even as it occurs on *Papaver*, for these two species are recorded as hosts in Europe. It is conceivable that some of the species that remained uninfected might prove susceptible under optimum conditions. These tests, although run in summer, were made in the greenhouse in order to minimize the risk of the disease spreading to the main plantings. Temperatures could be kept down only by lowering the light intensity considerably; and this fact, coupled with the tendency of Papaveraceae to be seriously checked by transplanting, was reflected in the finding that no conidia were formed on any plants, and that the teliospores were generally sparser, slightly smaller and thinner walled than is usual. The lesions on *P. somniferum* were, moreover, smaller than typical.

The problem of describing *Entyloma* species adequately for a reasonable segregation on morphological grounds is being discussed in another paper (7). The most useful characters are minimum and maximum length of teliospores, minimum and maximum width of teliospores, range of spore wall thicknesses, density of teliospore distribution, and presence or absence of conidia. The reader is referred to that paper for a fuller discussion of this subject and a consideration of the species concept in *Entyloma*.

Table I gives the essential data for a number of specimens of *Entyloma* on Papaveraceae. Several local collections on *Papaver* have been included, in order to indicate the amount of variation that may occur in a single strain of *E. fuscum*, for without such an indication one cannot assess the distinctness of forms on other host genera. The artificially inoculated material discussed above has been excluded from the table, since it is not regarded as entirely normal. The lesions, although fairly constant on any one host, vary considerably from plant to plant. The lesions are large and round on *Glaucium flavum*, moderately large and round on *Papaver Rhoeas*, and at first round on *P. somniferum*, but soon becoming large, irregular, and sometimes coalescent; on *Dendromecon* and *Eschscholzia* they are small and round. On *Papaver* the conidia are borne hypophyllously in a conspicuous mycelial web that seems to be derived mainly from the conidiophores but may also result to some extent from the germination of conidia *in situ*. It may be mentioned, in passing, that this web is a valuable field diagnostic character, for the bacterial leaf spot caused by *Xanthomonas papavericola* also produces round lesions on *P. Rhoeas* and large, irregular ones on *P. somniferum*. On *Glaucium*, *Dendromecon*, and *Eschscholzia* the conidia are borne amphigenously; they are abundant on *Glaucium* and *Eschscholzia*, but are sparse on *Dendromecon*, sections of the latter showing only one to four conidiophores in a stoma.

In Flora Hungarica Exsiccata 603, large spherical cysts, up to  $30\mu$  in diameter, occupy many parenchyma cells in the invaded areas of the leaf. In freshly made mounts these bodies are very conspicuous and show pro-

TABLE I

A COMPARISON OF *Entyloma* SPECIES ON VARIOUS SPECIES OF PAPAVERACEAE

Specimen	Host	Teliospore dimensions ( $\mu$ )			Teliospore distribution	Conidia
		Length	Width	Wall		
Vesterg. Micr. Rar. Sel. 874, <i>E. Glaucii</i>	<i>Glaucium flavum</i>	9.2-17.0	8.5-14.0	1.0-3.0 (3.5)	3	A
DAOM 14369*	<i>Papaver orientale</i>	10.0-18.0	8.5-14.0	0.5-2.5	2	—
DAOM 14370	<i>P. Rhoeas</i>	9.6-20.0**	8.2-15.9	0.7-3.2	3	H
DAOM 15438	<i>P. Rhoeas</i>	11.3-16.7	10.8-14.0	0.2-0.7	1	H
DAOM 15439	<i>P. somniferum</i>	10.5-20.0	9.5-16.0	1.0-4.3	3	H
DAOM 15440	<i>P. somniferum</i>	11.2-19.5	10.7-16.0	0.7-4.0	3	H
Flora Hungarica Exsiccata. 603	<i>P. somniferum</i>	10.2-21.5	10.0-15.0	0.7-3.0	3	H
Reliquiae Farlowianae, 693	<i>P. ? somniferum</i>	10.0-17.0	9.6-15.3	0.7-2.5	3	H
Baker Pac. Coast Fungi, 225, <i>E. Eschscholtziae</i> (ex. G. L. Zundel)	<i>Dendromecon</i> sp.	9.2-18.5	7.5-12.7	< 0.2	4	A
Univ. Calif. Herb. 405153, <i>E. Eschscholtziae</i> (per G. L. Zundel)	<i>Eschscholtzia californica</i>	8.7-16.0	7.2-11.5	0.5-1.2 (1.6)	4	A

Note: Name given under specimen is that under which it was issued; where no name is given, the specimen was issued as *E. fuscum*. Under teliospore distribution, 1 = sparse, 2 = moderately abundant, 3 = abundant, 4 = crowded. Under conidia, A = amphigenous, H = hypophyllous, — = absent.

\* DAOM is the identifying symbol of the Mycological Herbarium of the Dominion Department of Agriculture.

\*\* In stem lesions spores in this material ran up to 30  $\mu$  in length.

nounced radial striations. Some of the material of which they are composed is, however, soluble in lactophenol, and after a few hours the central portion largely disappears and the striations in the outer part become fainter. Fig. 5 shows the appearance of such a cyst after partial dissolution. Similar, but much smaller, cysts, up to 12  $\mu$  in diameter, were seen in Vestergren's *Micromyces Rariores Selecti* 874. In this material the bodies dissolved almost completely soon after mounts were made. Similar cysts have been seen in cells of *Thalictrum dioicum* infected by *Entyloma Thalictri*. These bodies appear to be encysted haustoria, but they differ markedly in composition from those often encountered in the rusts, which, as shown by Thatcher (8), are essentially similar in composition to the spore walls.

The smut on *Glaucium flavum* resembles *Entyloma fuscum* in size, wall thickness, and distribution of teliospores, and differs only in the manner in which the conidia are borne. Liro is unquestionably correct in including this fungus with *E. fuscum*, particularly in view of the close relationship of *Glaucium* and *Papaver*. Admittedly the fungus on *Glaucium* may be specialized upon that host, but probably such specialization is of no higher order than that which is suspected to occur within the smut on *Papaver*. No material of *E. Fragosi* on *G. corniculatum* has been seen, but it seems to have been segregated by Ciferri principally on the basis of the appearance of the lesions

on the two species of *Glaucium*. Without supporting experimental evidence such a separation is entirely untenable. The statement by Ciferri (1), in connection with his study of *E. fuscum* on *Papaver* spp., that the macroscopic appearance is the most important differential character, is invalidated by the finding that a single strain of the fungus produces entirely different lesions on *P. Rhoeas* and *P. somniferum*.

### ***Entyloma Eschscholtziae* Harkness**

The disposition of the material on *Dendromecon* and *Eschscholzia* is considerably more difficult than of that on *Glaucium*. *Entyloma Eschscholtziae* Hark. was described from material on *Eschscholzia californica* and is apparently confined to California; the fungus on *Dendromecon* was later assigned to it (by Farlow, as the information on the label of Baker Pacific Coast Fungi 225 indicates) with considerable justification in view of the morphological similarity of the fungi, the close relationship of the hosts, and the geographic distribution. Conidia were not noted in the description of this species, and their discovery in these two specimens diminishes the distinction between it and *Entyloma fuscum*. The remaining differences are a slightly smaller spore size, a thinner spore wall than is usual in *Entyloma fuscum* and a greater density of spores in the leaf. If these differences prove to be constant, *Entyloma Eschscholtziae* should probably be maintained. The segregation receives some support from the host relationships and geographic distribution. The fungus on *Dendromecon* has, in the specimen examined, extremely thin spore walls, but this difference alone does not merit specific distinction. It should be noted that DAOM 15438, collected in June, 1945, on *P. Rhoeas*, has smaller, sparser, and thinner-walled teliospores, and generally smaller lesions, than the several collections made in July and August, 1944, of which DAOM 14370 is typical. The spores measured seem, however, to be mature, and the difference may be due to the specimen having been collected after a long spell of cold, wet weather that may have adversely affected the host. That such a variation can occur emphasizes the risk of basing segregations on minor morphological differences unless such differences have been shown, by the examination of abundant material, to be constant. It is hoped that further collections on *Dendromecon* and *Eschscholzia* will be forthcoming in order that the position of these forms may be better assessed.

### **Conclusion**

For the present, at least, the smut on *Dendromecon* and *Eschscholzia* is to be regarded as a distinct species. The fungus on *Glaucium* should, however, be included with *Entyloma fuscum*: careful measurements of the extremes of spore lengths and widths and of spore wall thickness show no appreciable differences; and in both host genera we find a similar spore distribution pattern and abundant conidia.

Summarizing the data for the various specimens examined, the two species may be described briefly as follows:—

*Entyloma fuscum* Schroet. Teliospores generally abundant in the host tissue,  $9.2-20.0$  ( $21.5$ )  $\times$   $8.2-16.0\mu$  (up to  $30\mu$  long in stem lesions), wall  $(0.2) 0.5-3.0$  ( $4.3$ )  $\mu$ ; conidia amphigenous on *Glaucium*, hypophyllous and generally in a conspicuous mycelial web on *Papaver*.

*Entyloma Eschscholtziae* Hark. Spores crowded in host tissue,  $8.7-18.5 \times 7.2-12.7\mu$ , wall less than  $1.5\mu$ ; conidia amphigenous, abundant on *Eschscholzia* and sparse on *Dendromecon*.

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# BROWN POCKET ROT OF SITKA SPRUCE<sup>1</sup>

BY J. E. BIER<sup>2</sup> AND MILDRED K. NOBLES<sup>3</sup>

## Abstract

A brown pocket rot of Sitka spruce (*Picea sitchensis* Carr.) occurs in stands on the Queen Charlotte Islands, B.C. The decay, the sporophore associated with it, and the cultural characters of the fungus are described. The causal fungus is regarded as a new species and is described as *Lentinus Kauffmanii* sp. nov. by Dr. Alexander H. Smith, University of Michigan.

In 1942 the attention of the senior author was directed to a decay in Sitka spruce, known locally as "pocket rot," which was encountered in certain areas in the Queen Charlotte Islands frequently enough to make it of concern to the logging companies. The disease was reported to occur in patches, destroying the merchantable contents of many of the largest trees that would otherwise have yielded logs of the superior quality urgently required for aircraft construction. Further, it was stated that there appeared to be no external indications by which the presence of this decay in living trees could be recognized and allowed for in cruises. This resulted in some doubt being cast on the accuracy of the inventories prepared for standing timber, especially in accessible small areas that contained only a limited number of large trees suitable for sawlog production.

A pocket rot in Sitka spruce was described briefly by Mounce (1, p. 22) in the Report of the Dominion Botanist for the year 1926. Specimens of the decay and cultures isolated from it in the Dept. of Agr., Ottawa, Mycological Herbarium and stock culture collection show that it was identical with the rot under investigation. Mounce reported the isolation of the fungus causing this decay from freshly sawn Sitka spruce in British Columbia and also from specimens of Sitka spruce exhibiting "dote," removed from lumber received in England from British Columbia. These cultures remained unidentified until the present study.

Examination of the literature pertaining to the rots of Sitka spruce and correspondence with pathologists in the United States failed to disclose any additional references to a decay of the pocket rot type. Consequently investigations were undertaken to determine the incidence and economic importance of the decay, the causal organism, and, if possible, some criterion by which the disease could be recognized in standing trees.

<sup>1</sup> Manuscript received May 16, 1946.

Contribution No. 872 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

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## The Disease

### *Geographical Distribution*

During the course of this investigation brown pocket rot was found to be generally distributed in the Queen Charlotte Islands. Cultures of the causal fungus have been isolated from infected trees in Alaska, Washington, and Oregon. Dr. Alexander H. Smith has reported in correspondence that sporophores identical with those produced by the fungus causing brown pocket rot have been collected from logs of Sitka spruce in California. It appears, therefore, that the fungus has a wide distribution that probably coincides with the range of Sitka spruce.

### *The Decay*

The decay is a brown pocket rot of the heartwood of living Sitka spruce trees. It may occur as a butt, trunk, or top rot, but it was observed most frequently as a butt and trunk rot confined to the first and second 40-ft. logs, with an occasional infection extending into the third log (Figs. 3, 4). Given sufficient moisture, the fungus may continue to develop in dead trees for long periods. Sporophores of the causal fungus were collected on infected trees that are known to have been felled more than 50 years ago.

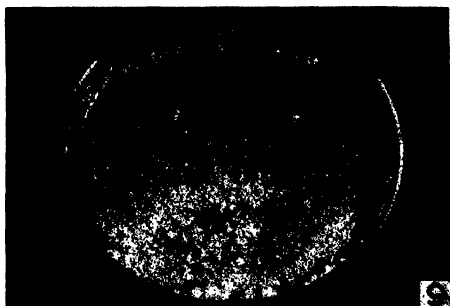
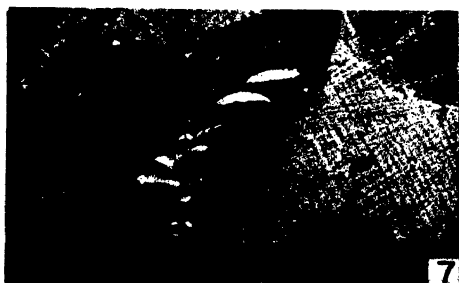
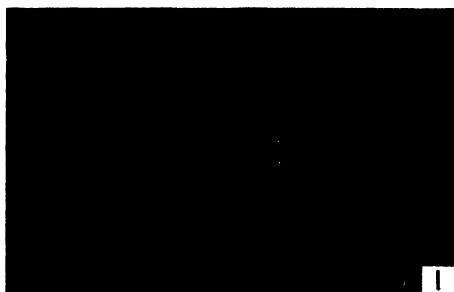
In the incipient stage of decay a faint brownish discoloration develops in minute pockets in the wood (Fig. 1). Later the pockets increase in size and become filled with a dark brown crumbly mass that tends to break into cubes, frequently with plates of white mycelium in the cracks. This decayed wood usually drops out of the centre of the pocket leaving large holes edged with rot. The individual pockets are broadly lens-shaped, ranging in size from 12 to 24 in. in length by 4 to 8 in. in width at the middle of the pocket, being elongated in the direction of the grain. They are sharply delimited from the apparently sound wood surrounding them (Figs. 2, 3, 4). In the final stages the pockets may become so numerous that they unite into a continuous column of rot (Fig. 3). It is common to find isolated pockets at some distance from the main body of decay and separated from it by what appears to be sound wood, although it has been shown by cultural methods that the fungus may be present throughout. Because of this distribution of pockets the amount of rot visible on the two ends of a log may be no indication of the extent of internal decay. The difficulty in estimating for brown pocket rot has resulted in scalers culling most logs showing this defect.

### *Occurrence and Method of Infection*

In a general survey of the decays in Sitka spruce in the Queen Charlotte Islands, 1977 trees, from different localities, including both slope and bench

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*Lentinus Kauffmanii*. FIG. 1. Incipient stage of brown pocket rot. FIG. 2. Individual pocket in longitudinal section.  $\times 0.125$  approx. FIG. 3. Brown pocket rot on the stump end of a basal log. FIG. 4. Decay 40 ft. along the log from Fig. 3. FIG. 5. Brown pocket rot in association with scar. FIG. 6. Fruit bodies on Sitka spruce.  $\times 0.5$  approx. FIG. 7. Fruit bodies on the cut surface of an infected log. FIG. 8. Fruit bodies on an infected log that had been on the ground for a period exceeding 50 years. FIG. 9. Culture on malt agar, six weeks old.







forest types, were analysed. Brown pocket rot occurred on all the types investigated, being responsible for 91 infections, or 6.4% of the total of 1414 infections in the trees examined. It ranked sixth in the list of decay-producing fungi on the basis of number of infections. The disease could not be regarded as serious in any locality nor was it found to occur solely in patches, causing the total loss of all trees in isolated areas, as had been claimed.

Since the disease frequently occurs as a root and butt rot and was reputed to be destructive in patches, it was thought that infection might be largely through the roots, by contact either with diseased roots or with the causal organism living saprophytically in the soil. However, it was observed that roots served as avenues of entrance for only 56% of the infections, while 36% were associated with scars, 3% with branch stubs, and 5% with dead tops. These latter types of infection, presumably initiated by air-borne spores, resulted in trunk and top rots in isolated trees. This further refutes the popular belief that the disease infects all the trees within a group.

The study failed to demonstrate a relationship between age of the tree and incidence of brown pocket rot. A number of trees in the younger age classes had become infected through scars caused by falling trees (Fig. 5).

### *Importance*

The gross merchantable volume (from stump height to an 11-in. top) of the 1977 trees analysed amounted to 13,968,223 bd. ft. (British Columbia Log Rule\*), of which 11.5% was destroyed by decay. Brown pocket rot resulted in a loss of 0.9% of the gross merchantable volume and was the fourth most important decay, following in order of importance conk rot (*Fomes Pini* (Thore) Lloyd), brown butt rot (*Polyporus Schweinitzii* Fr.), and brown crumbly rot (*Fomes pinicola* (Sw.) Cke.).

Since brown pocket rot occurs commonly as a root and butt rot it was of interest to determine its importance in Grade 1 logs, which are cut from the basal region of trees and which have the greatest value for the recovery of clear lumber. The total volume scaled† as Grade 1 in this work amounted to 5,119,267 bd. ft., of which decay had destroyed 7.2%. Brown pocket rot was responsible for the loss of 1.6% of the total volume Grade 1, and was second in importance to brown butt rot (*Polyporus Schweinitzii*) in logs of this grade.

Analyses of trees with basal infections demonstrated that when a large area of rot occurred in the stump end of the basal 40-ft. log, the decay extended into the second log (Figs. 3 and 4). Infections with a small number of isolated pockets at the base usually ran out before the top end of the first log.

No information is available on the persistence of the organism causing brown pocket rot in wood in service. Four cultures submitted for identifica-

\* Province of British Columbia. Log Scale. Victoria. 1944.

† The scaling and grading was undertaken in accordance with the standard practice outlined in the following publications:

Province of British Columbia. Forest Act. Victoria. 1941.

Province of British Columbia. Scaling lessons. Dept. of Lands, Victoria. Revised 1942.

tion that proved to be this species were labelled as having been isolated from "Sitka spruce lumber," one from a mill in Alaska, one from a mill in Oregon, and two from British Columbia. It is probable that they had been obtained from freshly cut lumber. At least two specimens of this decay were encountered in 1925 and 1926 by Dr. Irene Mounce in her study of samples of defective Sitka spruce lumber that had been removed from shipments received in England. In these the pockets were minute, and according to a letter, dated Mar. 18, 1926, from Dr. E. J. Butler of the Imperial Bureau of Mycology, who forwarded the specimens, the defect could not "be detected on the uncut log and only on the finished spars with very close examination". No further records of its occurrence in worked wood are known.

#### *External Indications of Decay*

One of the aims of the study was to find some external indication by which the presence of brown pocket rot in standing trees could be recognized. In some species of wood-rotting fungi the fruit bodies are produced on the living trees and these provide a reliable proof of the presence of the decay within the tree. In the case of the organism producing brown pocket rot, the fruit bodies are formed exclusively on infected wood that has been exposed to the atmosphere. Hence they have not been found on the bark of standing trees nor on the ground adjacent to them, and neither fruit bodies nor any other outward symptoms have been observed that would indicate the presence of brown pocket rot in standing trees.

### **The Fungus**

#### *Sporophores*

Small agarics were found associated with pocket rot in recently felled trees (Fig. 6), at the margin of pockets of advanced decay on the ends of logs (Fig. 7), and in abundance in the exposed pockets in old logs (Fig. 8). Cultures isolated from the rots and from spores and context of the associated fruit bodies proved to be identical. Hence it was concluded that these were fruit bodies of the organism causing the decay.

Specimens of the sporophores were submitted to Dr. Alexander H. Smith, University of Michigan, for identification. Dr. Smith replied that he had examined previous collections of the fungus and considered it to be an undescribed species, and he kindly consented to describe it for presentation here.

#### *"Lentinus Kauffmanii* A. H. Smith sp. nov.

"Pileus 3-8 cm. latus, convexus dein late convexus vel planus, saepe lobatus vel ad marginem undulatus, siccus, demum glaber, pallidus dein subincarnato-alutaceus; lamellae confertae, adnatae vel lineato-decurrentes, angustae, pallidae vel sordide pallido-incarnatae, serrulatae; stipes 3-6 cm. longus, 5-12 mm. crassus, aequalis, solidus, centralis vel eccentricus, siccus, pallide subincarnato-alutaceus; sporae  $5-6 \times 2\mu$ ; pleurocystidia  $60-100 \times 7-12\mu$ , subcylindrica vel fusioide ventricosa. Specimen typicum legit A. H. Smith n. 8520, prope Fort Dick, Calif., Nov. 8, 1937; in Herb. Univ. of Mich. et Dept. of Agr., Ottawa, Myc. Herb., conservatum.

"Pileus 3-8 cm. broad, convex, the margin at first incurved, becoming broadly convex to plane and in age the margin frequently elevated, wavy and irregularly lobed, surface dry and at first hoary from a thin cottony covering, soon appearing glabrous and unpolished, whitish from the conspicuous sheen but soon becoming sordid pale incarnate-alutaceous,

when mature the colors varying from pale buff to dull tan with scarcely any pinkish cast visible; flesh tough and cartilaginous, dull pinkish to sordid pinkish buff, odor and taste merely fungoid; lamellae crowded, bluntly adnate but decurrent by long ridges which extend down the stipe for 1–2 cm., narrow to moderately broad, equal, whitish to pale pinkish buff (4), edges finely serrate; stipe 3–6 cm. long, 5–12 mm. thick, equal, solid, central or eccentric, at times nearly lateral, concolorous with the pileus and with the same canescent covering at first, flesh pinkish, base white-felty mycelioid.

"Spores  $5-6 \times 2\mu$ , subcylindric to slightly curved, smooth, hyaline, not amyloid; basidia  $30-34 \times 5-6\mu$ , four-spored, pale reddish in iodine; pleurocystidia very abundant,  $60-100 \times 7-12\mu$ , hyaline, subcylindric to somewhat ventricose, often quite ventricose before elongating; cheilocystidia very abundant,  $60-125 \times 5-9\mu$ , the apex usually enlarged slightly and the central portion equal; gill trama homogeneous, scarcely colored in iodine, of narrow sub-parallel to somewhat interwoven hyphae, the hyphae broadest at the cross walls and with abundant clamps; pileus trama homogeneous beneath a turf of more or less upright slender filaments  $4-5\mu$  in dia. (these almost form a palisade), no lactifers seen; stipe with numerous hyphae projecting from the surface but not as crowded as over the pileus.

"Scattered to gregarious on spruce logs and stumps along the Pacific Coast from California to the Queen Charlotte Islands. The type was collected at Fort Dick, Calif.

"*Observations*: This species is readily distinguished by the absence of a veil, the very small almost allantoid and non-amyloid spores, conspicuous cystidia, the structure of the cuticle of the pileus and the pale sordid buffy incarnate colors. Kauffman collected it on several occasions in the Pacific Coast area and had considered it undescribed. He found it growing within old logs or in dark places on the under sides of trunks piled up as the result of wind storms. The type, however, was found scattered all over a fallen spruce trunk. Kauffman in his notes compared it with *L. umbilicatus*, but it can be readily distinguished from that species by its lack of an acrid taste, its spores, and the plane to only broadly depressed instead of umbilicate pileus. It is very closely related to *L. adhaerens* (Alb. et Schw.) Fr. but differs in lacking the viscosity of that species, in having smaller spores, and in lacking any appreciable odor and taste. In color, stature, habitat, cystidia and non-amyloid spores it is similar to *L. adhaerens* as described by Konrad et Maublanc."

In this study an abundance of sporophores was found during the months of May, June, September, and October. Invariably they developed from the wood at the margin of pockets that had become exposed to the atmosphere. The stumps, roots, and ground surrounding the bases of infected trees were examined carefully for the presence of fruiting bodies, but in no instance were they found.

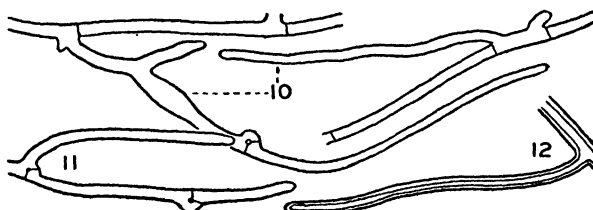
### Cultures

In the course of this investigation, 35 isolates of *Lentinus Kauffmanii* have been studied in culture. Of these, four were obtained from either the context or spores of fruit bodies of *L. Kauffmanii* and in each case a culture for comparison was isolated from the accompanying rot. These cultures, whether from rot or associated sporophore, proved to be identical, which established beyond question that *L. Kauffmanii* is the fungus causing brown pocket rot. The remaining cultures were isolated from decays and were identified by comparison with the authentic cultures from fruit bodies.

Each isolate was subjected to the routine procedure followed in the Ottawa Laboratory for the identification of fungus cultures, described by the junior author in earlier publications (2, 3). It was observed that cultures of this species, irrespective of their source, are remarkably uniform and readily recognizable. Furthermore they remain constant, three cultures isolated in 1925 being still identical with the newest isolates. A description of the cultures, based on the 35 isolates referred to above, follows.

### Growth Characters

Growth moderately rapid to slow, plates covered in four to five weeks (Fig. 9). Advancing zone even, hyaline, and appressed in zone up to 1 cm. broad, the mycelium so scanty that limit of growth can be determined only by removing lid of Petri dish and examining surface of colony by reflected light. Mat white at first and remaining so or developing pinkish buff (4) to cinnamon buff areas after five to six weeks, appressed, downy to thin woolly, with numerous scattered compact dots or nodules, of which the surface is felty to smooth and skin-like, ranging from 1 mm. or less in diameter on young colonies up to 5-10 mm. on six weeks old cultures. Reverse unchanged. Odour strong, disagreeable. On gallic and tannic acid agars diffusion zones lacking or weak, no growth on either medium.



*Lentinus Kauffmanii*. FIG. 10. Hyphae from advancing zone. FIG. 11. Regularly nodose-septate aerial hypha. FIG. 12. Fibre hypha.  $\times 600$ .

### Hyphal Characters

*Advancing zone*: leading hyphae hyaline, thin-walled, with inconspicuous simple septa, frequently branched, the branches soon developing clamp connections, 2.2-4.5  $\mu$  diameter (Fig. 10). *Aerial mycelium*: (a) nodose-septate hyphae as described above; (b) fibre hyphae numerous in older cultures, the walls thick and refractive, lumina narrow or apparently lacking except at tips, occasionally branched, 2.2-3.0  $\mu$  diameter (Figs. 11, 12). *Submerged mycelium*: (a) nodose-septate hyphae as described above; (b) crystals numerous, large, octahedral.

The nodulose surface of the older cultures on Petri dishes and in culture tubes is characteristic and distinct in appearance from any other species in the culture collection. The mycelium of the advancing zone in which hyphae with simple septa give off branches bearing clamp connections, is typical, and is found in the cultures of relatively few species of wood-destroying fungi. Normal sporophores have been produced occasionally on older cultures in tubes, but their formation is too rare to be of diagnostic value. However, even without fruit bodies, it should be possible to recognize cultures of *Lentinus Kauffmanii* readily.

### Acknowledgments

The authors wish to thank Dr. Alexander H. Smith for his kindness in examining the fruit bodies and preparing the description of the new species, and Mrs. G. Stewart (Dr. Irene Mounce) for permission to use her notes and photographs.

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## COLIFORM CONTAMINATION OF EGGS<sup>1</sup>

BY E. H. GARRARD<sup>2</sup>

### Abstract

Bacteriological examination of 1080 eggs from pullorum infected hens showed 78 (7.2%) to be contaminated with coliform organisms, which were classified into various types. No coliform organisms were isolated from 1000 eggs laid by hens free from pullorum disease.

### Introduction

During the past two years an increasing number of coliform isolations have been made at this laboratory from ovaries, oviduct, and liver of hens from pullorum infected flocks. When the opportunity of examining a considerable number of eggs from pullorum infected hens was presented in connection with another project (3), the writer decided to determine whether any of the infected birds laid coliform contaminated eggs. If such contamination were present, it might be a factor in the occasionally reported coliform counts in liquid, frozen, and dried eggs (10, 2, 17).

There is no lack of literature concerning contamination of eggs, but there is disagreement as to how and when organisms enter the egg. In support of the theory that organisms enter the yolk before the shell is laid down, Pernot (18) and Hadley and Caldwell (8) maintained that infection of the normal ovary is possible and Lamson (13) and others have questioned the sterility of the oviduct. Rettger (19) presented the opposite view and found no organisms in 200 samples of normal ovaries examined. According to Maurer (14) who made extensive examinations of both the interior and exterior of eggs from various sources, coliform organisms were absent from the interior of the eggs but were present on the surface of all dirty eggs liberally covered with faeces. It is generally agreed that yolks are more heavily contaminated than egg whites and numerous claims have been made as to the bactericidal properties of the latter (15, 20, 22). Maurer (14), Hadley and Caldwell (8), and Pennington (16) have reported varying degrees of contamination of egg yolk, and Rettger (19) isolated coliform types from yolks on 43 occasions during extensive investigations.

While workers have failed to agree as to whether ovaries may be infected with coliform or other types (other than pathogenic forms), or whether the yolks may become contaminated while passing through the oviduct, it is generally admitted that contamination after laying is responsible for the majority of bacteria within the egg. It has been proved that under certain conditions, organisms can penetrate the shell, and high coliform counts are

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*Contribution from Department of Bacteriology, Ontario Agricultural College, Guelph, Ont. This work was assisted in part by a grant from the National Research Council.*

<sup>2</sup> *Professor and Head of Department.*

generally the result of handling stored, dirty, and cracked eggs. With the exception of some types of rots such as "sour" eggs, where coliform organisms may be in the majority (9, p. 543) it is doubtful whether they play an important part in egg spoilage. Moreover, Johns (11), analysing coliform counts in powdered egg, indicated that there was little correlation with plate and microscopical counts of viable organisms or the result of sanitary surveys. However, while the coliform group is still regarded as the index of faecal contamination, the presence of *Escherichia coli* and allied types cannot altogether be ignored.

### Experimental

#### *Source of Eggs*

The eggs from pullorum infected hens were obtained from a flock of 55 hens comprised of Barred Rocks, Light Sussex, New Hampshires, and Leg-horns. They were procured from various flocks in Ontario that had previously been tested for pullorum disease by the tube agglutination test. Most of them were either constant or intermittent reactors to both standard and variant (24) pullorum antigens, while a few exhibited reactions of a suspicious or non-specific character. The hens were mixed in several pens in a well ventilated, up-to-date chicken house, situated half a mile from the laboratory. They were tested weekly and the eggs collected daily. Because of lack of facilities, the hens were not trap-nested, nor were the eggs graded. Although an attempt was made to test the eggs as they were received, occasionally a storage period of a few days was necessary.

To compare the results with eggs from non-diseased hens, eggs were also obtained from College flocks in which there were no pullorum reactors. These eggs were ungraded and were treated exactly the same as the eggs from the pullorum infected flock.

#### *Methods of Analysis*

As the isolation of types and not numbers was the primary object, only qualitative tests were conducted, nor was there any attempt made to distinguish between possible contamination of yolk and white. The method of analysis followed was somewhat similar to that described by Gibbons, Moore, and Fulton (6). The eggs were inspected for cracks and, if sound, were soaked in a 500 p.p.m. chlorine solution, after which they were rinsed in a 60% alcohol solution and placed in a sterilized, perforated rack to dry. The ends were painted with a strong solution of iodine and the eggs again allowed to dry. Using aseptic precautions, the ends were punctured with a flamed awl, and the contents of the egg were blown, by compressed air blown through sterile glass wool, into a sterile, wide-mouthed flask containing broken glass. Each flask was shaken for at least three minutes after which 1- and 2-ml. amounts of egg meat were transferred in triplicate tubes of lactose brilliant green bile broth. If positive after incubation for 48 hr. at 37° C., transfers were made to eosin methylene blue plates, colonies were picked off after suitable incubation, and the isolates typed according to methods previously described (5).

## Results

A total of 1080 eggs from pullorum infected hens was examined during the months of July, August, and September. From 78 eggs, cultures were isolated that produced gas in brilliant green bile broth and that proved, on microscopical examination, to be Gram negative, short rods. By using the classification adopted by the Ministry of Health, Appendix I, Standard Methods (1), which is based upon I. M. Vi. C. reactions, sucrose fermentation, hydrogen sulphide production, and gelatine liquefaction, the cultures proved to be members of the coliform group. The results are shown in Table I.

TABLE I  
CHARACTERISTICS OF 78 CULTURES ISOLATED FROM EGGS LAID  
BY PULLORUM INFECTED HENS

No. of cultures	Type	I. M. Vi. C.	No. of cultures fermenting sucrose	No. of cultures liquefying gelatine	No. of cultures producing H <sub>2</sub> S	No. of cultures fermenting lactose at 44° C.
52	<i>E. coli</i> , Type I	+ + - -	8	0	0	41
8	<i>E. coli</i> , Type II	- + - -	0	0	0	0
0	<i>A. aerogenes</i> , Type I	- - + +	0	0	0	0
8	<i>A. aerogenes</i> , Type II	+ - + +	8	0	0	0
8	<i>A. aerogenes</i> , Intermediate Type I	- + - +	1	0	7	0
2	<i>A. aerogenes</i> , Intermediate Type II	+ + - +	1	0	1	0

Because of claims of various workers (4, 21, 23) that fermentation of lactose at 44° C. assists in further separating faecal *Escherichia coli* from allied types, this method was included. It will be noted that approximately 80% of the *E. coli* Type I cultures fermented lactose at that temperature while the *Aerobacter aerogenes* and Intermediate forms showed no sign of fermentation at 44° C.

From these same eggs, in connection with another project (3), 61 cultures of *Salmonella pullorum* were isolated. In only six cases, however, were cultures of *S. pullorum* and coliform organisms isolated from the same egg.

Analysis of 1000 fresh eggs during the months of October, November, and December, obtained from hens negative to pullorum disease, failed to produce a single coliform culture. While it has been claimed that higher counts of organisms are found in eggs during the summer months (13) other work would indicate that such is not the case (18) and it is not thought that the time of year greatly influenced the results with regard to types of coliform organisms isolated.



## Discussion

While there is no way of proving that coliform contamination in eggs from pullorum infected flocks was not derived from sources outside of the egg, the negative results from eggs from healthy hens, housed and attended under approximately the same conditions, make it fairly conclusive that the organisms were localized in the hens. It still remains to be definitely proved that coliform organisms readily contaminate the interior of the eggs from infected straw, faecal deposits on the shell, etc., and recent work by Johns and Bérard (12) would indicate that such is not the case.

Because the birds were not trap-nested, it was not possible to determine whether any one hen consistently laid coliform contaminated eggs. Few workers record the isolation of coliform organisms from the ovaries at post-mortem where isolations of *S. pullorum* have been made. When the hens in question were posted, most of the coliform isolations obtained were from those hens that gave suspicious or non-specific reactions to the agglutination test, and from those that yielded *S. pullorum* variant strains from ovaries severely affected. However, the fact that in six eggs both *S. pullorum* and coliform organisms were isolated is an indication that both organisms may be present at times in the ovaries or oviduct and that the pullorum reactor may be responsible for laying coliform contaminated eggs.

Although such contamination may be significant with regard to certain spoilage and in adding coliform counts to egg products, of greater significance to laboratories engaged in annually testing thousands of birds for pullorum disease is the possibility of coliform and allied types of organisms being responsible for suspicious and non-specific reactors. Post-mortem examinations of hens reacting weakly or non-specifically to both the tube and rapid agglutination tests have revealed, on numerous occasions, nothing but coliform types, not only from the ovaries and oviduct but from the liver and spleen. Similar findings have been reported elsewhere (7). It is apparent that a considerable amount of work is necessary before the true significance of such organisms can be determined.

## Acknowledgments

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# CARBOHYDRATE METABOLISM OF MCINTOSH APPLES DURING THEIR DEVELOPMENT ON TREE AND IN COLD STORAGE

## PART 1. GENERAL TRENDS<sup>1</sup>

BY G. KROTKOV<sup>2</sup> AND V. HELSON<sup>3</sup>

### Abstract

For two consecutive years, weekly samples of apples were taken during the development of fruits on the tree and in subsequent cold storage. Following determination of their respiration, the fruits of each sample were analysed for various forms of carbohydrates and also for their insoluble *P* content. The last constituent was assumed to represent the 'protoplasmic factor.' Six different physiological stages are distinguished during the ontogeny of apple fruits. These are based on changes in the direction and in the rates of metabolism of various carbohydrates. The first three stages are passed while apples are on tree; the next one is passed either partly or even completely in storage, depending on the date of picking; the last two are passed entirely in storage. At the beginning of storage there was observed a sharp increase in the sugar content of apples. Since there was no corresponding decrease in the alcohol insoluble residue, it was concluded that such sugars must come from some alcohol soluble substances.

### Introduction

It was shown previously (9), that when an apple fruit is placed under standardized conditions and its respiration is followed for a period of time, the kind of respiration record produced will depend on the ontogenetic stage of the fruit in question. In the early stages of fruit ontogeny, respiration records are represented by the preclimacteric periods only. In the middle stages there appears a climacteric rise dividing the whole record into the preclimacteric and postclimacteric periods. In later stages the preclimacteric periods disappear completely, and the records obtained are represented by the postclimacteric periods only. It was also shown that from the beginning of July and onward the bulk of the carbon dioxide was produced in the post-climacteric period, and that the duration of life of a fruit was now directly proportional to the length of this particular period.

It has been suggested that the climacteric rise in respiration might be due to an increase in the concentration of the respiratory substrate. This last increase is brought about by the acceleration of the hydrolytic processes in an apple at this stage of its ontogeny. If this suggestion is correct, it may be assumed that the respiratory rate of a fruit from now on is determined primarily by the concentration of the respiratory substrate, and the duration of its life by the amounts of the reserve substances from which this substrate is produced. Since several forms of carbohydrates are present in an apple

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fruit in large amounts, one might expect that some of these do serve as such reserves. It was decided, therefore, to investigate the carbohydrate metabolism of apples, and this paper presents the results of such investigations.

### Materials and Methods

The work was carried out in 1941-42 and 1942-43, using McIntosh apples as experimental material. In 1941-42 all the samples studied came from the same tree, which also supplied all the apples used in the previous work (9). In 1942-43, owing to a series of unfavourable circumstances, the experimental material was not so homogeneous. The first four samples were taken from one tree in a small private orchard near Kingston. All the subsequent samples until the end of December were supplied by another tree in the same orchard. The keeping quality of the apples in 1942-43 was not good, the percentage infected with apple scab was high, and so by the end of December this source of supply was exhausted. A bushel of apples was then purchased from a large commercial orchard about 75 miles from Kingston. This was placed in cold storage, and all the remaining samples were taken from this stock.

Throughout both growing seasons apple samples were taken directly from the tree. In the fall several bushels of apples were picked from the same tree, placed in cold storage at 2° C., and these were the apples that supplied the subsequent samples.

Each year the first sample of apples was taken as soon as the petals fell, which was taken to be the time of the fruit setting. In 1941-42 the first sample taken on May 31st consisted of 341 apples. The next few samples contained progressively smaller numbers of apples, until from July 10th and onward each sample consisted of three apples. In 1942-43 the first sample taken on June 2nd had 334 apples, and after progressive decline in the number of apples in a sample, all the samples taken from July 20th and onward consisted of five to six apples each.

Samples of apples were brought to the laboratory, weighed, and their respiration, expressed as carbon dioxide production, was determined at 2° C. for 24 hr. Determinations of the carbon dioxide production were made by the continuous gas method, using Pettenkoffer tubes as described earlier (9).

After determination of their respiration the apples were frozen at -14° C. Early samples consisting of small apples were frozen *in toto*. Samples of larger apples taken in 1941-42 after July 3rd and in 1942-43 after July 13th were first peeled, cored, quartered, and then frozen. While in a frozen state the apples were passed through a meat grinder, and the pulp so obtained was thoroughly mixed. It was this pulp that was used for the carbohydrate and insoluble *P* analyses.

For the carbohydrate analyses, 12 to 15 gm. of the frozen apple pulp were extracted with 300 cc. of 85% ethyl alcohol under a reflux condenser for 24 hr. The alcoholic filtrate of such an extract was used for the determination of the

sugars, while the alcohol insoluble residue was analysed for starch and hemicelluloses. The alcohol was removed from the alcoholic filtrate under reduced pressure at a temperature below 30° C. The clearing of the sugar extracts, and their decolorization by boiling them with charcoal was done as recommended by Archbold (1). The sugar solutions finally obtained were inverted with hydrochloric acid, using the slow method of inversion (3, p. 373, Sec. 23 (c)). The only change introduced into this method was the extension of the period of hydrolysis from 24 to 48 hr. Hanes' modification (4) of the Hagedorn Jensen method was used for the determination of the reducing power of the extracts. Glucose and fructose were determined in the extracts before inversion, using Widdowson's technique (12), which is based on a combination of the alkaline ferricyanide and hypiodite oxidation. The only modification introduced into this method was that hypiodite oxidation was done at 2° C. and not at 1° C. All the results of sugar analyses were expressed as percentage of sugar per fresh weight of apple pulp.

Starch was extracted from the alcohol insoluble residue according to the method described by Hanes (5) by "solubilizing" it with hydrochloric acid in 95% ethyl alcohol while it was being boiled for 12 min. under a reflux condenser. The residue was then filtered off, suspended in distilled water, brought to the boiling point, and the starch was extracted by further digestion for 20 min. in a boiling water-bath. The aqueous extract was made up to volume, an aliquot was inverted with hydrochloric acid (3, p. 373, Sec. 23 (c)) and analysed for reducing power. The results were calculated as glucose and expressed either as percentage of apple pulp, fresh weight, or of the alcohol insoluble residue.

Following extraction of the starch, the alcohol insoluble residue was further digested for three hours in a boiling water-bath with 1% sulphuric acid. The filtrate was freed of sulphuric acid by barium hydroxide, and, after neutralization, its reducing power was determined. The results were calculated as glucose and expressed either as percentage of apple pulp, fresh weight, or of the alcohol insoluble residue.

For the analysis of the insoluble *P* content of apples, 12 to 15 gm. of frozen apple pulp were refluxed for 15 min. in a mixture of 150 cc. of 95% ethyl alcohol and 50 cc. of ether to remove lipids. To extract its water soluble *P*, the apple residue was transferred to a beaker and suspended in 10 times its volume of freshly prepared 5% trichloroacetic acid. The suspension was stirred mechanically for 20 min., allowed to stand for two hours, filtered, and dried. The dry residue was placed in an envelope and stored in a refrigerator until the time of analysis.

Incineration of this residue was done in an electric furnace for three hours at 500° C. Phosphorus was determined in the ash according to the method described by King (8), using a Klett-Sommerson photoelectric colorimeter.\*

\* The authors wish to express their thanks to the American Academy of Arts and Sciences, which provided the funds necessary to buy the instrument.

The results were calculated as mgm. of  $P$  per 100 gm. of apple pulp, fresh weight.

In preliminary work, lipids and water soluble  $P$  were removed from several samples of apple pulp, and the residue was incubated further with 1% sodium hydroxide at 30° C. The filtrates obtained from such digests gave a negative test for  $P$ . Apparently apple pulp has no organic phosphorus-containing substances that are insoluble in a mixture of absolute ethyl alcohol and ether, which are precipitated with trichloroacetic acid, and from which their phosphorus can easily be split off by mild hydrolysis. The usual substances that answer this description are phosphoproteins. Another phosphorus-containing substance that is precipitated with trichloroacetic acid, but from which  $P$  cannot be released with ease by digestion with 1% sodium hydroxide, is nucleoprotein. The analyses for the insoluble  $P$  have been carried out, therefore, on the assumption that the amounts of such  $P$  found in an apple may serve as a measure of the nucleoprotein content. In still more general terms, the insoluble  $P$  serves as a measure of the 'protoplasmic factor.' While several objections may be raised against such an assumption, it is interesting to examine the data with this in mind.

## Results

Figs. 1 and 2 present changes in the respiration and insoluble  $P$  content of apples in 1941-42 and 1942-43, respectively. Changes in respiration are of the type reported and described earlier (7, 9). In both years and particularly

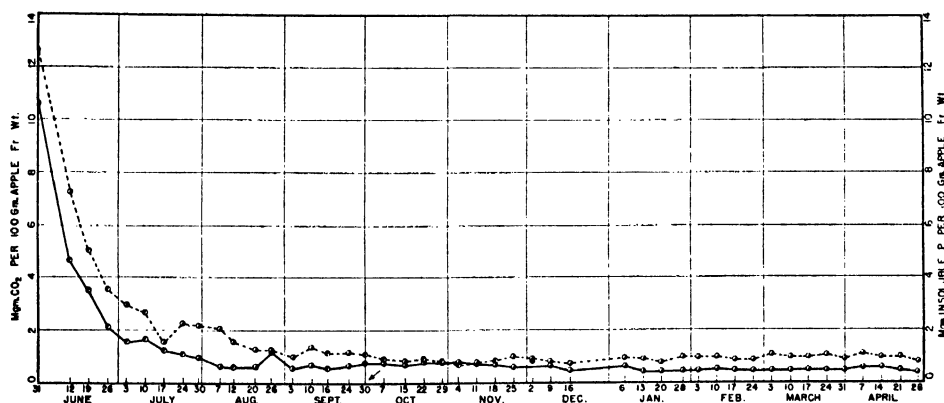


FIG. 1. Respiration and insoluble  $P$  content of apples in 1941-1942.

●—● respiration, ● - - - ● insoluble  $P$ , ↓ date of picking.

in 1942-43 the climacteric rise observed was very small. This is probably due to the fact that respiration was determined at 2° C., and as reported by Smock (11) climacteric is much more evident at high temperature than at low.

The insoluble  $P$  content of the apples was high initially, and it dropped down very rapidly during the first month of growth. In subsequent months it continued to decline at a progressively slower rate, reaching low values by

the beginning of October in 1941-42, and by the end of August in 1942-43. For the rest of the ontogeny these low values were maintained with slight fluctuations.

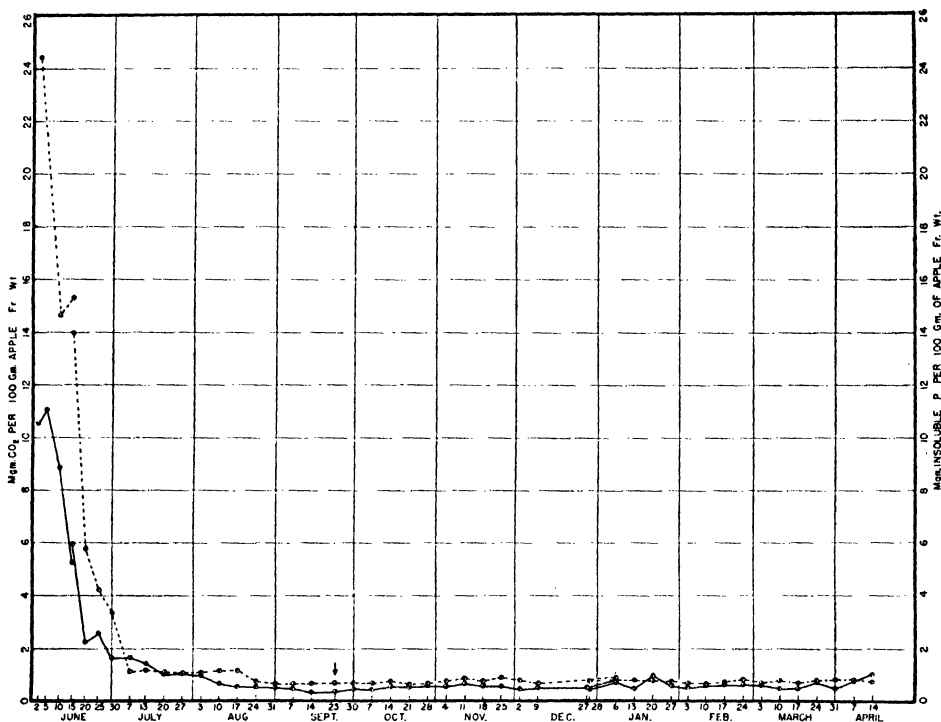


FIG. 2. Respiration and insoluble *P* content of apples in 1942-1943.

●—● respiration, ●-----● insoluble *P*, ↓ date of picking.

Close parallelism between the changes in the insoluble *P* content and the respiration of apples supports the original assumption, that the insoluble *P* content of an apple may be taken as a general measure of the 'protoplasmic factor.'

Figs. 3 and 4 give data on changes in various sugars. Examination of these figures reveals that during the development on the tree the total sugar content of an apple was continuously increasing. This increase was at first rapid and then it slowed down. Toward the end of September or early in October there was another rapid rise, reaching the peak of 12.5% in 1941-42 and 10% in 1942-43. After that there was a steady decline for the rest of the storage.

In both years, following a rapid increase within the first two weeks of the season, the glucose content became practically constant for a long time, with a tendency toward a slight increase in the last period of storage. The fructose content was continuously increasing, at first rapidly, then at a slower rate. Having reached a high value, towards the end of October it dropped for a while, and then began a steady rise for the rest of the storage life.

In 1941-42 after more or less steady values for the first two weeks, the invert sugars began to increase at first slowly and then more rapidly. The peak was reached early in October, after which time there came a decline at first

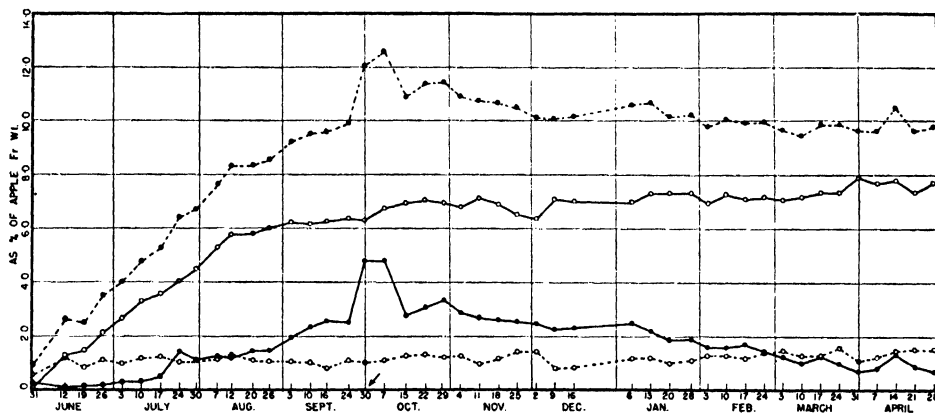


FIG. 3. Sugar content of apples in 1941-1942.

○ — glucose, ○ — fructose, ● — invert sugars, ● — total sugars, ↓ date of picking.

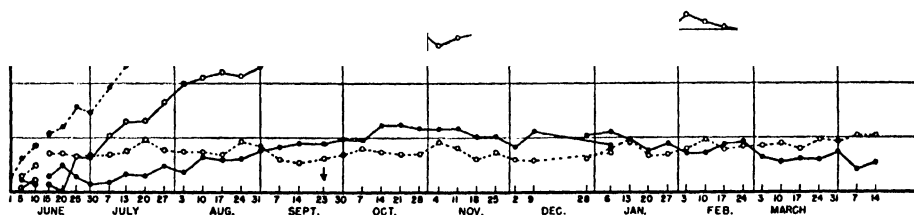


FIG. 4. Sugar content of apples in 1942-1943.

○ — glucose, ○ — fructose, ● — invert sugars, ● — total sugars, ↓ date of picking.

rapid and then slow throughout the rest of the storage life. In 1942-43 after the initial fluctuations around a low value for about a month, invert sugars began to increase, coming to a peak by the middle of October. Having reached the peak, there followed a continuous, steady decline throughout the rest of the observed life.

Figs. 5 and 6 give data for the seasonal changes in the starch content of apples. Starch was found in apples from the very time of their setting. The amounts present initially were small, and, expressed per fresh weight of an apple, they were declining during the first few weeks. From the middle



of June, however, starch began to accumulate, reaching in 1941-42 a single peak by the first week in August, and in 1942-43 a double peak toward the end of July and by the middle of August. Having reached its peak, it began

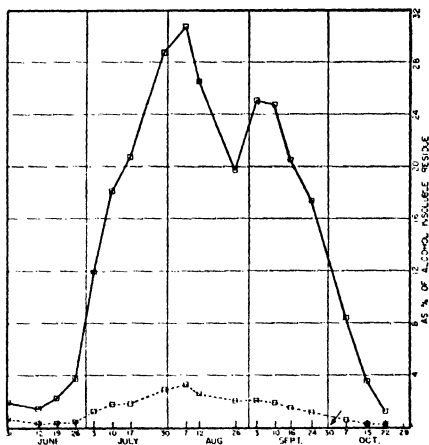


FIG. 5

FIG. 5. Starch content of apples in 1941-1942.

□ - - - - - □ as % of the apple fresh weight, □ — — — — □ as % of the alcohol insoluble residue, ∇ date of picking.

FIG. 6. Starch content of apples in 1942-1943.

□ - - - - - □ as % of the apple fresh weight, □ — — — — □ as % of the alcohol insoluble residue, ∇ date of picking.

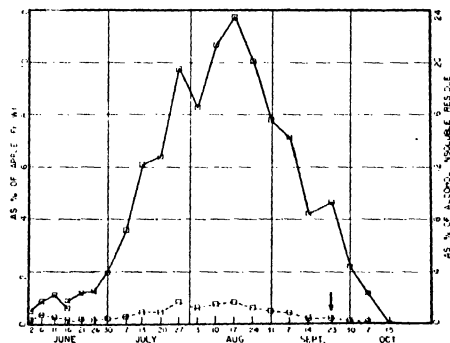


FIG. 6

to disappear, and the last traces of it were gone in 1941-42 by the end of October, and in 1942-43 by the middle of the same month. If starch is expressed as percentage of the alcohol insoluble residue, then its seasonal changes are much more rapid. At its peak, starch formed over 30% of such a residue in 1941-42 and 24% in 1942-43.

Figs. 7 and 8 present changes in the hemicellulose content of apples. In both years early in the season the hemicellulose content, as expressed per apple pulp, fresh weight, was high. It declined rapidly at first, slower later, reaching steady values by the end of October in 1941-42, and by the middle of September in 1942-43. From this time and onward these values were maintained with a very slow and steady decline for the rest of the storage.

If expressed per alcohol insoluble residue, the hemicellulose content appears to be at first sight remarkably constant throughout the whole ontogeny. Fluctuations observed appear to be around an average value of about 8%, which was the same in both years. A careful examination of these fluctuations reveals, however, that their order and timing were very close in both years. Thus in June the percentage of hemicelluloses in the alcohol insoluble residue was steadily increasing, coming to a peak by the end of the month. Throughout July it declined, reaching low values early in August. Then followed another peak, which in 1941-42 was a small one and of short duration, while

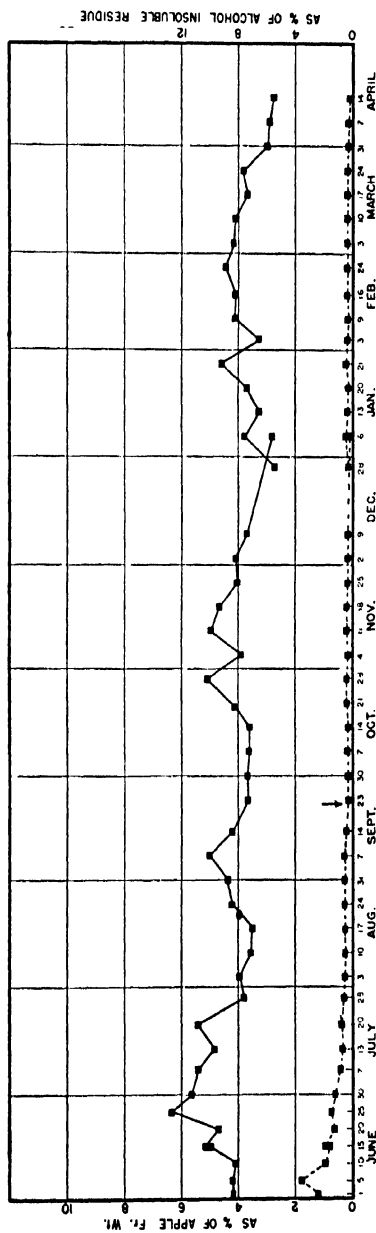
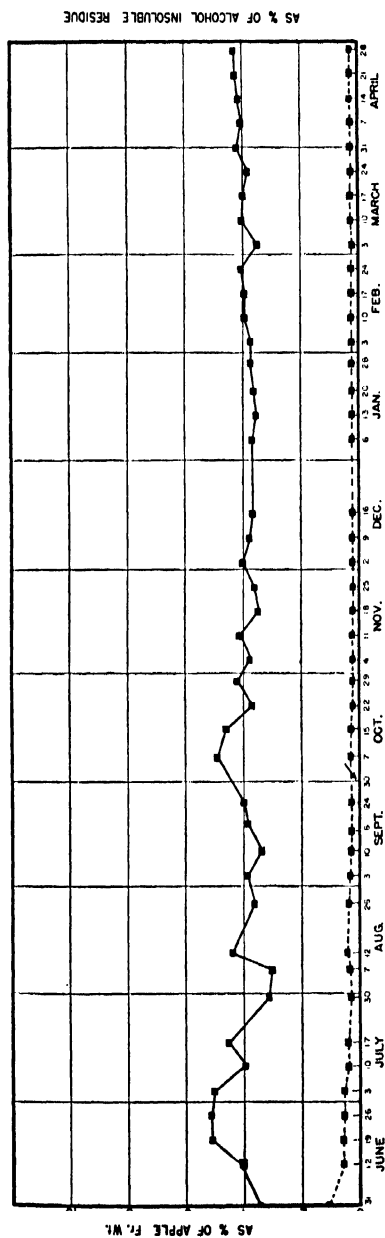


FIG. 8

Fig. 7. Hemicellulose content of apples in 1941-42.

—○— as % of the apple fresh weight, —■— as % of the alcohol insoluble residue.

Fig. 8. Hemicellulose content of apples in 1942-43.

—○— as % of the apple fresh weight, —■— as % of the alcohol insoluble residue.

in 1942-43 it was larger and lasted longer. The second largest peak of ontogeny occurred in October, and this was followed by a long and steady decline. From January and onward there was another slow rise.

### Discussion

Examination of Figs. 3 to 8 reveals that during its ontogeny an apple fruit passes through a series of successive stages. These are characterized by different concentrations of various carbohydrates as well as by different rates and directions of their metabolism. A transition from one stage to another is indicated by an inflection in a corresponding graph. Six such stages can

TABLE I

THE DURATION AND RATES OF CHANGES IN DIFFERENT CARBOHYDRATES DURING VARIOUS STAGES OF FRUIT ONTOGENY, 1941-42\*

—	Total sugars	Glucose	Fructose	Invert sugars	Starch	Hemi- celluloses
<b>Stage 1</b>						
Duration	May 31 to June 12 or 19	May 31 to June 12	May 31 to June 12 or 19	May 31 to June 26	May 31 to June 12	May 31 to June 12
Days	15	12	15	26	12	12
Rate in mgm./day	+108	+52.1	+81.2	-1.5	-15.3	-42.8
<b>Stage 2</b>						
Duration	June 12 or 19 to Aug. 12	Constant	June 12 or 19 to Aug. 12	June 26 to Aug. 12	June 12 to Aug. 7	June 12 to Oct. 22
Days	58		58	47	56	132
Rate in mgm./day	+99		+76.4	+20.8	+26.2	-2.9
<b>Stage 3</b>						
Duration	Aug. 12 to Sept. 24	Constant	Aug. 12 to Sept. 30	Aug. 12 to Sept. 24	Aug. 7 to Oct. 29	
Days	43		49	43	83	
Rate in mgm./day	+38		+10.7	+30.2	-19	
<b>Stage 4</b>						
Duration	Sept. 24 to Oct. 7	Constant	Sept. 30 to Oct. 22	Sept. 24 to Oct. 7		
Days	13		22	13		
Rate in mgm./day	+207		+34.8	+176		
<b>Stage 5</b>						
Duration	Oct. 7 to Dec. 9	Constant	Oct. 22 to Dec. 2	Oct. 7 to Dec. 9		
Days	63		41	63		
Rate in mgm./day	-39.5		-17.9	-39.8		
<b>Stage 6</b>						
Duration	Dec. 9 to Apr. 28	Dec. 9 to Apr. 28	Dec. 2 to Apr. 28	Dec. 9 to Apr. 28		
Days	140	140	147	140		
Rate in mgm./day	-2.4	+3.5	+9.8	-11.8		

\* Rates have been calculated from changes in the concentrations of different carbohydrates expressed as percentage of apple pulp, fresh weight.

be distinguished. The first three are passed while the apple is attached to the tree. The fourth stage is passed either partly or completely in storage, depending on the time of picking. The last two are passed entirely in storage. In 1941-42 the apples were picked after they entered the fourth stage of their ontogeny, and in 1942-43 they were picked before this stage. The duration of each stage and the mean rates of change of the several carbohydrates at each stage are summarized in Tables I and II. Owing to a change in the source of experimental material by the middle of the winter of 1942-43, no comparable data could be computed for the sixth stage of this year.

TABLE II

THE DURATION AND RATES OF CHANGES IN DIFFERENT CARBOHYDRATES DURING VARIOUS STAGES OF FRUIT ONTOGENY, 1942-43\*

—	Total sugars	Glucose	Fructose	Invert sugars	Starch	Hemi-celluloses
Stage 1						
Duration	June 1 to June 25 or 30	June 5 to June 15	June 5 to June 25 or 30	June 5 to July 7	June 5 to June 15	June 1 to June 20
Days	27	10	22	32	10	20
Rate in mgm. day	+92.7	+81.3	+49	-2.8	-24.7	-26
Stage 2						
Duration	June 25 or 30 to Aug. 10	Constant	June 25 or 30 to Aug. 24	July 7 to Aug. 24	June 15 to Aug. 17	June 20 to Sept. 14
Days	44		58	48	63	86
Rate in mgm./day	+89.5		+51.6	+18	+11.2	+5.3
Stage 3						
Duration	Aug. 10 to Sept. 30	Constant	Aug. 24 to Sept. 23	Aug. 24 to Oct. 7	Aug. 17 to Oct. 14	
Days	51		30	44	58	
Rate in mgm./day	+41.5		+62.6	+15.2	-13.8	
Stage 4						
Duration	Sept. 30 to Oct. 21	Constant	Sept. 23 to Oct. 21	Oct. 7 to Oct. 21		
Days	21		28	14		
Rate in mgm./day	+49		+5.9	+40.5		
Stage 5						
Duration	Oct. 21 to Dec. 2	Constant	Oct. 21 to Nov. 25	Oct. 21 to Dec. 2		
Days	43		35	42		
Rate in mgm./day	-28.4		-19.8	-19.4		

\* Rates have been calculated from changes in the concentrations of different carbohydrates expressed as percentage of apple pulp, fresh weight.

The first stage begins at the time of the fruit setting, and it lasts for two or three weeks. During this stage the concentrations of the total sugars are low, although the rate of their increase is the highest for any period of time while the apples are still attached to the tree. It is also the only stage in the life of an apple when the concentration of glucose is higher than that of

fructose. Both of these sugars are rapidly increasing, and by the end of this stage their concentrations become equal. Invert sugars are practically constant. Both starch and hemicelluloses are progressively decreasing, and while concentrations of starch are low, those of hemicelluloses are the highest of all the carbohydrates.

In the second stage the rate at which the total sugars increase remains high, being only slightly lower than that in the first stage. From now, and until the last physiological stage in storage is reached, the glucose content remains practically constant, varying around the value reached at the end of the first stage. Fructose is increasing at practically the same rate as in the first stage. Invert sugars, which up to now have been practically constant, begin to accumulate. The most apparent characteristic of this stage is the rapid increase in the starch content of the apples. In 1941-42 this was taking place at a rate practically twice that of 1942-43.

In the third stage the rate of increase of the total sugars dropped in both years to a value slightly less than half of that in the preceding stage. With the glucose content still constant, this increase was due to a rise in both fructose and invert sugars. There was, however, a fundamental difference in the accumulation of these two sugars in both years. In 1941-42 the main increase was due to invert sugars. The rate of fructose accumulation in this year dropped to a value about one-seventh of that for the preceding stage, while the rate of increase of the invert sugars was up 50%. In 1942-43 the main increase was due to fructose. It is this last sugar that accumulates now at an even greater rate than in the second stage, while the rate for the invert sugars accumulation is somewhat decreased. In both years starch began to disappear. In 1941-42 the rate of its disappearance was lower than that of its accumulation in the previous stage. In 1942-43 these two rates were about the same.

In the fourth stage the rate for the accumulation of total sugars goes up again, and the highest concentrations of the total and invert sugars during the whole ontogeny of an apple occur at the end of this stage. The rate of increase in total sugars, as compared with that for the preceding stage, was increased five times in 1941-42, and only slightly in 1942-43. In both years this increase in total sugars was due mainly to the accumulation of invert sugars and to a lesser extent to fructose.

In the fifth stage there is observed for the first time a decrease in the total sugar content of the apples, and this decrease is shared by both invert sugars and fructose. In 1941-42 the rate of disappearance of invert sugars was about twice that of fructose. In 1942-43 the rates of decrease for both sugars were about equal.

In the last or sixth stage of ontogeny the glucose content, which, in Stages 2, 3, 4, and 5 has been more or less constant, now begins a slow and steady increase. The decrease in total and invert sugars, observed in the preceding stage, is continued. On the other hand, concentrations of fructose, which were declining in the preceding stage, from now on begin to increase.

As is seen from Tables I and II, different stages have various durations even for the same carbohydrate. Thus the first and fourth stages are the shortest, and the sixth is the longest. Neither is the duration of the same stage identical for different carbohydrates. Thus in 1942-43 the length of the first stage varied for various carbohydrates from 10 to 32 days. Variations in the duration of various stages were also observed from one year to another. For all these reasons, taking an apple fruit as a whole, one could give only approximate values for the duration of its successive physiological stages.

While Tables I and II indicate the directions and the rates of changes in the concentrations of various carbohydrates, they do not reveal the magnitude of the changes involved. To get a picture of the absolute amounts of various sugars that were either added or removed from a single apple during any one of these stages, one should take into consideration the rate of changes, the length of the period during which this rate was operative, and the simultaneous increase in the weight of an apple. In order to bring this to light the data obtained have been recalculated as follows. In both years the average weights of single apples were plotted against the time of taking samples, and through these points smooth curves were drawn. The graphs obtained were of the usual sigmoid type. From these graphs it was possible to determine for each year the weight of an average apple at any time of the growing season. Having assumed for each year the average lengths of various stages from the graphs for the total sugars, as summarized in Tables I and II, it was possible to calculate the changes in the fresh weight of a single average apple, as well as in the absolute amounts of various substances that were either added or lost in various stages of ontogeny. The results obtained are given in Tables III, IV, and V.

It is seen from Table III that the final weight of an average apple in 1941-42 was considerably larger than in 1942-43. In both years, however, the greatest proportion of the apple's final weight was produced during the second stage of growth, and it was also in the same stage that the mean daily increment in the weight of an apple was the highest. The third stage, with its mean daily increment only slightly lower, was responsible for practically all the remaining final weight of an apple. Only small additions were made during the first, and when late picking permitted, during the fourth stages. In both years the mean daily increment, expressed as percentage of the initial weight of an apple, was the highest in the first stage, and it was progressively declining. In other words, the relative growth rate of an apple is highest in the first and lowest in the last stages of growth.

In 1941-42 the greatest increase in the total sugar content of an average apple occurred in Stage 2. Somewhat smaller amounts were added during the third stage and approximately 25% during the fourth. In 1942-43 the greatest increase in the total sugars was in the third stage. The next greatest increase was in the second, and finally only 10% was added in the fourth stage. In 1941-42 the mean daily increments for the total sugars were highest in the fourth stage. They were two and a half times higher than those for the

TABLE III  
CHANGES IN THE WEIGHT OF AN AVERAGE APPLE

—	1941-1942			1942-1943		
	Fresh weight in gm.	As % of the final wt.	As % of the initial wt.	Fresh weight in gm.	As % of the final wt.	As % of the initial wt.
Stage 1	May 31 to June 12 or 19, 15 days			June 1 to June 25 or 30, 27 days		
At the beginning	0.17			0.1		
At the end	3.00			7.0		
Added	2.83	2.2		6.9	7.2	
Mean daily increment	0.189		111	0.255		255
Stage 2	June 12 or 19 to Aug. 12, 58 days			June 25 or 30 to Aug. 10, 44 days		
At the beginning	3.00			7.0		
At the end	80.00			53.0		
Added	77.00	59.4		46.0	48.5	
Mean daily increment	1.330		44.2	1.042		14.8
Stage 3	Aug. 12 to Sept. 24, 43 days			Aug. 10 to Sept. 30, 51 days		
At the beginning	80.00			53.0		
At the end	123.00			95.0	100	
Added	43.00	33		42.0	44.3	
Mean daily increment	1.000		12.5	0.822		1.6
Stage 4	Sept. 24 to Oct. 7, 13 days					
At the beginning	123.00					
At the end	130.00	100				
Added	7.00	5.4				
Mean daily increment	0.538		0.44			

preceding stage. In 1942-43 these increments did not increase in the fourth stage as compared with the third but were decreased by half.

In both years it was during the second stage that the greatest amounts of glucose were added to an apple. In 1941-42 the bulk of fructose was also added in the second stage, being closely followed by the third, and the final 10% was added in the fourth. In 1942-43 the greatest increase in fructose was during the third stage. There was a smaller increase in the second, and the final 10% was added in the fourth stage as before. While in 1941-42 the mean daily increments for the fructose accumulation in the second, third, and fourth stages were about the same, in 1942-43 they varied, and there was a considerable drop in such an increment during the fourth stage.

In 1941-42 the highest amounts of invert sugars were added during the fourth stage and in 1942-43 during the third. In both years the mean daily increments of invert sugars in an apple were continuously increasing from the first to the fourth stages. However, in 1941-42 there was a fivefold increase in this increment during the fourth stage as compared with the third while in 1942-43 the increase was only a slight one.

TABLE IV

INCREMENT PER AVERAGE APPLE AND MEAN RATES OF CHANGE OF VARIOUS  
CONSTITUENTS PER APPLE PER DAY 1941-1942

	Total sugars	Glucose	Fructose	Invert sugars	Starch	Hemi- celluloses	Alcohol insoluble residue less starch and hemi- celluloses	Insoluble P
Stage 1	May 31 to June 12 or 19, 15 days							
Added in mgm. per apple	+75	+31	+39	+5	+3	+16	+145	+0.162
As % of the total amount added	0.5	2	0.5	0.1	0.3	4.4	4.8	11.7
Mean daily increment in mgm.	+5	+2	+2.6	+0.36	+0.2	+1.05	+9.6	+0.0108
Stage 2	June 12 or 19 to Aug. 12, 58 days							
Added in mgm. per apple	+6573	+1039	+4585	+949	+996	+310	+2270	+1.05
As % of the total amount added	40	72.9	52.5	15.2	99.7	85.5	75.5	77.5
Mean daily increment in mgm.	+113	+17.9	+79	+16.4	+17.2	+5.32	+39	+0.0180
Stage 3	Aug. 12 to Sept. 24, 43 days							
Added in mgm. per apple	+5550	+220	+3215	+2115	-323	-20	+540	+0.15
As % of the total amount added	34	15.3	36.6	34	32.3	5.5	17.7	10.8
Mean daily increment in mgm.	+129	+5.1	+74	+49	-7.5	-0.46	+12.6	+0.0035
Stage 4	Sept. 24 to Oct. 7, 13 days							
Added in mgm. per apple	+4200	+140	+910	+3150	-369	+56	+60	-0.22
As % of the total amount added	25.5	9.8	10.4	50.7	36.9	15.6	2	15.9
Mean daily increment in mgm.	+322	+10.8	+70	+242	-28.3	+4.32	+4.6	-0.0170
Stage 5	Oct. 7 to Dec. 9, 63 days							
Added in mgm. per apple					-308			
As % of the total amount added					30.8			
Mean daily increment in mgm.					-14.7			

Though in both years the bulk of starch appeared in the second stage, small amounts of it were also synthesized in the first one. From Figs. 5 and 6, presenting changes in the starch content of apples expressed as percentage of the apple, fresh weight, one might draw the conclusion that in the first stage there was hydrolysis and not synthesis of starch. This is due to the disproportion at this stage between the concurrent mean rates of starch production and the increase in weight of an apple. The first rate is low while the second one is relatively high. In 1941-42 the absolute amounts of starch hydrolysed in the third, fourth, and fifth stages were about equal. In 1942-43, 80% of the starch was hydrolysed in the third stage and the remaining 20% in the fourth.



TABLE V

INCREMENT PER AVERAGE APPLE AND MEAN RATES OF CHANGE OF VARIOUS  
CONSTITUENTS PER APPLE PER DAY 1942-1943

	Total sugars	Glucose	Fructose	Invert sugars	Starch	Hemi- celluloses	Alcohol insoluble residue less starch and hemi- celluloses	Insoluble P
Stage 1	June 1 to June 25 or 30, 27 days							
Added in mgm. per apple	+209	+93	+86	+30	+12	+47	+335	+0.241
As % of the total amount added	2.2	7.1	1.4	1.3	2.9	27.4	17	37.7
Mean daily increment in mgm.	+9.5	+4.2	+3.9	+1.36	+0.55	+1.7	+12.4	+0.0089
Stage 2	June 25 or 30 to Aug. 10, 44 days							
Added in mgm. per apple	+3490	+692	+2150	+648	+406	+91	+1053	+0.335
As % of the total amount added	36.2	53.2	35.8	27.7	97.1	52.9	54.5	53.2
Mean daily increment in mgm.	+79.2	+15.7	+48.8	+14.7	+9.2	+2.1	+23.8	+0.0076
Stage 3	Aug. 10 to Sept. 30, 51 days							
Added in mgm. per apple	+4930	+515	+3253	+1162	-333	+5	+320	+0.058
As % of the total amount added	51.3	39.7	54	49.6	80	2.9	16.3	9.1
Mean daily increment in mgm.	+96.5	+10	+63.7	+22.8	-6.5	+0.1	+6.3	+0.0014
Stage 4	Sept. 30 to Oct. 21, 21 days							
Added in mgm. per apple	+990	-10	+510	+500	-84.5	+29	+240	
As % of the total amount added	10.3	0.7	8.8	21.4	20	16.8	12.2	
Mean daily increment in mgm.	+47	-0.5	+24.3	+23.7	-6.5	+1.39	+11.4	

In both years the bulk of hemicelluloses was formed in the second and to some extent in the first stages. Little change took place during the third stage, and a further slight increase was observed in the fourth. Similar observations on the accumulation of hemicelluloses in developing apples were made by Widdowson (13).

The greatest amounts of the alcohol insoluble residue less starch and hemicelluloses were added in the second stage, with progressively smaller additions during the third and fourth stages.

In 1941-42 the apples were picked after the beginning of the climacteric rise in sugars that is characteristic of the fourth stage. One might claim, therefore, that such a rise was due to an increased importation from the tree. In 1942-43 such a rise occurred after picking, and one has therefore to rule this explanation out. A rise in the sugar content of apples after picking has been reported by other workers also (1; 6, pp. 106-124; 10, pp. 70-75). We can conclude, therefore, that such a rise is not due to an importation from the tree but to the appearance of sugars from some substances already present in the apple at the time of picking.

Table VI presents changes in the total carbohydrate content of apples during different stages of storage. During the fourth stage of fruit ontogeny there is a considerable rise in the sugar content of apples, which in 1941-42 was more than twice that in 1942-43.

TABLE VI  
CHANGES IN THE TOTAL CARBOHYDRATE CONTENT OF APPLES DURING  
DIFFERENT STAGES OF STORAGE\*

	1941-42	1942-43
Stage 4		
Total carbohydrate content (total sugars, starch, hemicelluloses) at the beginning	10728	9312
Total carbohydrate content at the end	13134	10286
An addition of	2406	974
CO <sub>2</sub> produced during this period, expressed as glucose	163	168
Total	2569	1142
Stage 5		
Total carbohydrate content at the beginning	13134	10286
Total carbohydrate content at the end	10289	9043
A loss of	2845	1243
CO <sub>2</sub> produced during this period, expressed as glucose	631	380
Difference	2214	863
Stage 6		
Total carbohydrate content at the beginning	10289	
Total carbohydrate content at the end	9801	
A loss of	488	
CO <sub>2</sub> produced during this period, expressed as glucose	925	
Difference	437	

\* Expressed as mgm. of total carbohydrates per 100 gm. of apple, fresh weight. Owing to a change in the source of apples by the middle of the winter in 1942-43, data for the sixth stage in this year are omitted.

As seen from Tables IV and V, starch, hydrolysed during this stage, could account only for less than 10% of these sugars, and there was a simultaneous slight synthesis and not hydrolysis of hemicelluloses. The alcohol soluble residue less starch and hemicelluloses could not be the ultimate source of these sugars, since it was shown in the same tables that the amounts of such a residue were still increasing during this stage. This last observation is supported by other workers (1, 13).

One is forced, therefore, to conclude that the bulk of these sugars must come from some alcohol soluble substances, and three types of such substances may be tentatively suggested. First, they are substances that are precipitated from the extracts during clearing with lead. Second, they escape hydrolysis under the mild conditions adopted in this work. Third, these sugars are produced from some non-sugar substances.

The disappearance of sugars from apples during the fifth stage is two to three times in excess of their utilization in respiration. This suggests their transformation into some other forms that again escape analysis. Archbold and Barter (2) observed an excess of 17 to 30% in the amounts of carbon disappearing from stored apples in the form of total sugar and acids above amounts lost as carbon dioxide. In the sixth stage the disappearing sugars have contributed approximately one-half of the respiratory substrate, while the other half must have come from some other source.

The absolute amounts of sugars released during the fourth stage are considerable. In 1941-42 and 1942-43 they formed 25 and 10% of all the sugars observed in apples at the peak of their sugar content. They are represented by a mixture of invert sugars and fructose, with sometimes one and sometimes the other predominating.

Equally large amounts of sugars, also represented by a mixture of both invert sugars and fructose, disappear without any traces during the fifth stage. The amounts involved in such transformations are too large to be omitted. Elucidation of the nature of these substances, which give rise to sugars in the fourth stage and into which sugars are transformed in the fifth stage, is necessary for the understanding of the carbohydrate metabolism of apples.

Progressive changes observed during the ontogeny of apples can be summarized as follows. In the first stage of ontogeny, which begins at the time of petals falling, high and rapidly declining rates of respiration, coupled with the equally rapidly decreasing concentrations of the insoluble *P*, suggest a progressive decrease in the relative amounts of living matter. Expressed as percentage of the apple, fresh weight, the hemicellulose content declines very rapidly, while that of starch only slightly. Concentrations of total sugars rapidly go up, and such sugars are represented mainly by fructose and glucose. Invert sugars remain either stationary or even decline somewhat. The relative growth rate of the fruit is the highest of its whole ontogeny, though as a result of its small weight the absolute amounts of various substances added during this stage are very small.

In the second stage respiration and concentrations of the insoluble *P* decline further, though at a slower rate. Concentrations of total sugars expressed as percentage of the apple, fresh weight, are increasing at only a slightly lower rate than in the preceding stage. This time the increase is due mainly to fructose and to a lesser extent to invert sugars, while the glucose content remains constant. Starch concentrations are increasing, while those of hemicelluloses decline further, though at a much slower rate than in the preceding stage. The mean daily increment in the fresh weight of an apple is the highest for the whole ontogeny and close to one-half of the final weight of an apple is formed during this stage. The absolute amounts of various constituents added during this stage are high. The bulk of hemicelluloses, glucose, insoluble *P*, alcohol insoluble residue less starch and hemicelluloses, and practically all of the starch accumulate in this stage.

In the third stage respiration reaches its lowest value, and then it begins to go up in the climacteric rise. Concentrations of the insoluble *P* reach low steady values, which are maintained for the rest of the ontogeny. The rates of increase in the concentration of the total sugars drop to one-half of those for the preceding stage. This increase may be due mainly either to invert sugars, and in such a case an increase in fructose is low, or to fructose, and then the rate of increase for the invert sugars is low. Hydrolysis of starch is probably the most striking feature of this stage. The absolute increase in the weight of an apple, though less than in the preceding stage, is still considerable, and for this reason the absolute amounts of various sugars added are large. The alcohol insoluble residue less starch and hemicelluloses is still being added, though in small amounts, and the accumulation of insoluble *P* in an apple may be completed.

A climacteric rise in respiration observed to begin by the end of the preceding stage is now followed by a rise of sugars in the fourth stage. There is an increase in both invert sugars and fructose. Sometimes it is one sugar that accumulates to a greater extent and sometimes the other. Since small increases in the alcohol insoluble residue less starch and hemicelluloses, and also in the hemicelluloses themselves, are still observed during this stage, and since the starch hydrolysed could contribute less than 10% of the increase of total sugars, such a rise must be due to an appearance of sugars from some alcohol soluble substances. Respiration still continues to rise. Hydrolysis of starch, begun in the previous stage, may be completed. If this stage begins before the fruits have been picked, a slight increase in the weight of an apple may still occur.

In the fifth stage respiration reaches its peak and begins to decline. Total sugars begin to decrease, their fall being shared by both invert sugars and fructose. During this stage more sugars disappear from an apple than could be accounted for by respiration.

In the sixth stage a further decrease in the total sugars now occurs at the expense of invert sugars only. Fructose begins to accumulate again, and glucose, which for the four preceding stages was practically constant, begins a steady rise. The amounts of carbon dioxide produced in respiration are now considerably in excess of the amounts of sugars simultaneously lost from apples.

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## INHERITANCE OF VARIEGATION IN BARLEY<sup>1</sup>

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### Abstract

A strain of variegated barley that originated in a Saskatchewan field produces, on sowing, progeny of which approximately 90% are albino, the remainder striped or variegated with rare full green exceptions. In crosses, variegated ♀ × green ♂ produced 7 albino, 4 striped, and 11 green  $F_1$  plants. The reciprocal cross yielded 1 striped and 41 green  $F_1$  plants.  $F_2$  segregation approximated three green to one of all others (albino and variegated). From some  $F_2$  and  $F_3$  progenies it was inferred that two genetic factor pairs might be segregating. On that hypothesis the dominant hypostatic factor for variegation must be very unstable, mutating at a high rate to white. The peculiarities of inheritance may be explained also, however, on the basis of a combination of gene and (maternal) plastid inheritance. On this interpretation the plastids present in the egg affect the colour of the seedling that develops from it. When green plastids or proplastids are present in the egg, many of them, but not necessarily all, are induced to mutate if the white  $w$  gene is homozygous, but fewer if the  $w$  gene is heterozygous. If white plastids only are present in the egg it is probable that the seedling will be an albino regardless of gene content.

### Introduction

The inheritance of leaf variegation involving absence of chloroplastids from a part of the mesophyll is not always Mendelian. A large proportion of the reported cases of maternal inheritance involve variegated plants, e.g., *Mirabilis jalapa* (3), *Zea* (1), and *Triticum* (10, 13). Variegation induced by genes and inherited in Mendelian fashion is also known (2, 9). In a third group (5, 11), results are affected by maternal plastid inheritance, gene segregation, and plastid mutation.

Imai (8) suggested that self-reproducing plastids contain plastogenes. Mutation of plastogenes may be induced by certain nuclear genes, and automutation independent of nuclear genes may also occur. When only a portion of the plastids of meristematic, e.g. embryo, cells mutate, segregation of plastids may occur at successive cell divisions resulting in the characteristic green and white sectors. Plastid mutation may occur commonly only at certain stages of the life cycle, e.g. in germ cells or cells of young embryos.

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Imai (5, 6) found in variegated barley that a mutated gene induced plastid mutation. Variegated plants, homozygous for the recessive gene, produced, when selfed, offspring of which about 94% were variegated and 6% were albino. In crosses with green plants the  $F_1$  were green if the mother was green; green or variegated if the mother was variegated. The plastid content of the egg at the time of fertilization affected the phenotype of the hybrid. The  $F_2$  ratio of three green to one variegated indicated that only one gene pair was involved.

In 1940, variegated and albino plants were reported in a farmer's barley field in Saskatchewan. After growing two generations of striped plants, the farmer provided us with seed for use in an inheritance study. The striped plants gave a large proportion of pure white offspring, a small proportion of striped plants, and almost no normal green segregates. The inheritance of variegation obviously was not identical with that reported for barley by Imai (5 to 8). It was decided that the material should be studied cytologically and that reciprocal crosses of striped with green plants were needed to clarify the situation.

### Materials and Methods

Seed from variegated barley was space-sown in the cereal breeding nursery at University farm, Saskatoon. The seed from 19 variegated plants grown in the nursery was planted in the greenhouse in a central bed, artificially lighted at night. The light, moisture, and humidity were regulated to resemble field conditions. A second generation was grown both in the greenhouse and the nursery. In each generation the progeny of single plants were separately recorded for leaf colour: that is, whether green, white, or green and white striped. In addition, crosses were made between striped plants and plants of several standard varieties. The  $F_1$ ,  $F_2$ , and  $F_3$  plants were classified for variegation.

The material was studied cytologically. Sections of green, white, and striped leaves were cut after fixation in Craf's fixing solution. Most of these were cut at 15  $\mu$  and stained in Heidenhain's haematoxylin or in crystal violet with iodine. Freehand sections of fresh leaves were also studied. Aceto-carmine smears of pollen mother cells were prepared and studied from anthers fixed in alcohol-acetic mixture (3:1). Some of the smears were made permanent or semipermanent by mounting in diaphane. Chromosome counts were made and pairing relations observed.

It should be noted here that the leaves of the variegated barley plants have alternating bands of green and white of widely varying widths (Fig. 1). Completely albino leaves and spikes occur occasionally. The leaves are limper and softer to the touch than are ordinary green leaves of barley.

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FIG. 1. *Photograph of variegated barley leaves.*

PLATE I







## Results

The segregation shown by the progenies of the 19 striped plants grown in the nursery is given in Table I. The proportions of white, striped, and green varied in the different progenies as follows: white, from 75 to 93%; striped, from 7 to 24%, and green, from 0 to 3%. The averages for all the progenies were 84.3% white, 14.5% striped, and 1.2% green.

TABLE I

THE SEGREGATION OBSERVED IN THE PROGENIES OF STRIPED BARLEY PLANTS  
AFTER SELF-FERTILIZATION IN 1942

Parent plant No.	No. of seeds sown	No. of plants emerged	Number of plants classified as			Percentage of plants classified as		
			White	Striped	Green	White	Striped	Green
1	122	115	98	14	3	85.2	12.2	2.6
2	122	118	108	10	0	91.5	8.5	0.0
3	122	119	103	16	0	86.6	13.4	0.0
4	122	121	100	19	2	82.7	15.7	1.6
5	108	105	89	14	2	84.8	13.4	1.8
6	122	121	91	29	1	75.2	23.9	0.8
7	122	113	101	9	3	89.4	8.0	2.6
8	40	35	32	3	0	92.5	7.5	0.0
9	112	111	100	11	0	90.1	9.9	0.0
10	37	37	33	4	0	89.2	10.8	0.0
11	49	49	45	4	0	91.8	8.2	0.0
12	122	119	99	20	0	89.9	10.1	0.0
13	69	67	52	14	1	77.6	20.9	1.5
14	122	118	98	17	3	83.5	14.4	2.1
15	122	119	92	26	1	77.3	21.8	0.9
16	122	120	91	25	4	75.8	20.2	3.4
17	57	54	50	4	0	92.6	7.4	0.0
18	44	43	39	4	0	90.7	9.3	0.0
19	58	58	48	9	1	82.8	15.5	1.7
Totals		1742	1469	252	21	84.3	14.5	1.2

After two generations of inbreeding at the University, the striped plants produced a somewhat higher proportion of white progeny than was observed in the earlier generations as shown by the results given in Table II. The proportion rose from 84.3 to 93.1% whereas the proportion of striped plants dropped from 14.5 to 6.9% and the proportion of greens fell to zero.

In another test (Part 2 of Table II) the progeny of 17 striped plants selfed for two generations at the University consisted of 359 white and 25 striped plants, that is, 93% of whites and 7% of striped. These figures agree very closely with those of Part 1 of Table II.

In 1945 the progeny (Part 3 of Table II) of another group of individual striped plants separately recorded totalled 897 of which 815 or 90.9% were white, 81 or 9% were striped, and 1 or 0.1% were green. These proportions of white and striped plants are not significantly different from those given in Part 1 of Table II.

TABLE II

THE SEGREGATION OBSERVED IN THE PROGENY OF STRIPED BARLEY AFTER TWO GENERATIONS OF SELF-FERTILIZATION IN 1943 AND 1944

Plant No.	Number of plants	Number of plants classified as		Percentage of plants classified as	
		White	Striped	White	Striped
Part 1					
1	200	189	11	94.5	5.5
5	238	224	14	94.1	5.9
6	250	229	21	91.6	8.4
8	36	35	1	97.2	2.8
10	76	74	2	97.4	2.6
11	55	53	2	96.4	3.6
13	282	263	19	93.3	6.7
15	191	168	23	88.0	12.0
16	234	220	14	94.0	6.0
Totals	1562	1455	107	93.1	6.9
Part 2					
(17 plants)	384	359	25	93.0	7.0
Part 3					
(30 ± plants)	896	815	81	90.9	9.0
Totals	2842	2629	213	92.0	8.0

Seeds from different heads of individual striped plants gave widely fluctuating results. Typical progeny results are given in Table III. Head *d* of B245-4 gave 36 white and 10 striped whereas Head *h* gave 43 white and 1 striped. Similarly Head *a* of B246-3 gave 20 white and 10 striped as contrasted with Head *c*, which gave 29 white and 1 striped.

The  $F_1$  results of crosses made with plants of commercially used barley varieties are reported in Table IV. The results from reciprocal crosses were not identical, which suggested that direct plastid transmission from mother to offspring influenced the results.

It may be observed from Table IV that of 22  $F_1$  individuals from striped ♀ × green ♂, only 11 were green, the remainder consisting of four variegated and seven white. This suggests that if mutated white plastids are present in the egg the gene for green is unable to convert them to the green condition. The variegated individuals may have come from eggs having mixed plastids. Since, in the reciprocal crosses, one individual out of 42 was variegated, it is indicated that the gene for variegation even when heterozygous may induce occasional plastid mutation.

TABLE III  
HEAD PROGENIES FROM INDIVIDUAL STRIPED PLANTS GROWN IN 1945

Parental plant	Head	White	Striped	Green	Total
B245-4	<i>a</i>	27	5	0	32
	<i>b</i>	28	0	0	28
	<i>c</i>	25	5	0	30
	<i>d</i>	36	10	0	46
	<i>e</i>	26	4	0	30
	<i>f</i>	26	3	0	29
	<i>g</i>	26	2	0	28
	<i>h</i>	43	1	0	44
	Totals	237	30	0	267
	%	88.8	11.2	0.0	
B246-3	<i>a</i>	20	10	0	30
	<i>b</i>	29	2	1	32
	<i>c</i>	29	1	0	30
	<i>d</i>	44	2	0	46
	<i>e</i>	28	2	0	30
	<i>f</i>	30	1	0	31
	<i>g</i>	39	8	0	47
	Totals	219	26	1	246
	%	88.9	10.5	0.4	

TABLE IV  
THE  $F_1$  RESULTS OF CROSSES BETWEEN STRIPED PLANTS AND GREEN  
PLANTS OF COMMERCIAL VARIETIES

Cross	Number of plants		
	Green	Striped	White
Striped × Warrior	3	1	1
Striped × Himalayan	2	0	1
Striped × OAC21	0	1	3
Striped × Hannchen	4	0	2
Striped × Regal	2	2	0
Warrior × striped	3	0	0
OAC21 × striped	6	0	0
Hannchen × striped	15	1	0
Regal × striped	17*	0	0

\* One of these was listed as pale green.

The progenies of selfed  $F_1$  plants gave the results presented in Tables V and VI. The results in Table V show the  $F_2$  segregation of crosses of green ♀ × striped ♂. Application of the  $\chi^2$  test to the Hannchen × striped results gives a slightly better fit to a 12 : 3 : 1 than to a 3 : 1 ratio. The possibility of a main gene for green vs. white and a hypostatic dominant gene for striped vs. white is suggested. However the fact, proved by  $F_1$  results, that phenotype is not a certain indication of genotype in this material should be kept in mind.

TABLE V

THE  $F_2$  SEGREGATION OF CROSSES OF GREEN PLANTS (FEMALE)  $\times$  STRIPED PLANTS (MALE)

Cross		Description of $F_1$ plants	Distribution of $F_2$ plants			Fit to 3 : 1 ratio of green: others	
Female	Male		Green	Striped	White	$\chi^2$	$P$
Hannchen	Striped	10 green, 1 striped	147	24	8	4.844	.03
Regal	Striped	One green	4	3	1	2.667	.10
Regal	Striped	One pale green	0	6	0	17.000	.00
Totals (excepting pale green)			151	27	9	3.293	.05

Table VI shows the  $F_2$  segregation of crosses of inbred variegated plants used as females with green plants of standard commercial varieties as male parents. There is a good fit to a 3 : 1 ratio of green to others excepting for the cross variegated  $\times$  Regal. The  $\chi^2$  test for all results excepting those for variegated  $\times$  Regal gave a  $P$  of .70.

TABLE VI

THE  $F_2$  SEGREGATION FROM HYBRIDS BETWEEN INBRED STRIPED FEMALE PLANTS AND MALE PLANTS OF COMMERCIAL GREEN VARIETIES

Cross		$F_1$ plant	$F_2$ plants			Green vs others	
Female	Male		Green	Striped	White	$\chi^2$	$P$
Striped	Warrior	Green	47	6	10	0.053	.80
Striped	Himalayan	Green	166	24	20	1.841	.20
Striped	OAC21	Striped	42	13	7	1.742	.20
Striped	Regal	Green	22	13*	4	7.240	.01
Striped	Regal	Striped	22	10**	4	3.704	.05
Totals (except striped $\times$ Regal)			255	43	37	.220	.70

\* Of which three were described as "pale green."

\*\* Of which two were described as "pale green."

The  $F_2$  results of reciprocal crosses are similar in that both give a fair fit to a ratio three green to one of all others. The  $F_1$  and  $F_2$  results considered together suggest that green is partly dominant to the white or striping factor. On this interpretation the inbred variegated plants used in the original crosses are genetically like their sister albino plants homozygous for the recessive "white" gene. That striped  $F_1$  plants possess the factor for green is shown by their green  $F_2$  offspring.

The  $F_3$  segregation from selfed green  $F_2$  plants from the cross Regal (green)  $\times$  variegated is reported in Table VII. Of 48  $F_2$  plants classed as green, 10 bred true, 34 segregated to give approximately three green to one of all others, three gave no green offspring, and one gave two green to 13 of others. If the

TABLE VII

THE  $F_2$  SEGREGATION FROM GREEN  $F_2$  PLANTS FROM THE REGAL (GREEN)  $\times$  STRIPED CROSS

$F_2$ plant No.	Distribution of $F_2$ plants			Test for 3 : 1 ratio of green to others		Classification as to 3 : 1 fit*
	Green	Striped	White	$\chi^2$	$P$	
B324 2	13	0	0	4.333	.04	B
B324 6	9	6	1	2.750	.10	A
B324 9	13	3	0	0.333	.50	A
B324 14	9	4	1	0.857	.30	A
B325 4	10	3	2	0.555	.50	A
B325 6	0	0	16	50.000	.00	C
B325 9	13	2	0	1.088	.30	A
B325 12	14	2	0	1.333	.20	A
B325 13	10	6	0	1.333	.20	A
B325 14	16	0	0	5.333	.02	B
B326 3	11	2	3	0.333	.50	A
B326 4	0	2	14	50.000	.00	C
B326 6	11	4	0	0.022	.80	A
B326 7	10	5	1	1.333	.20	A
B326 9	10	4	1	1.333	.20	A
B326 10	15	0	0	4.947	.02	B
B326 12	0	1	15	50.000	.00	C
B326 15	12	3	1	0.000	1.00	A
B327 1	12	3	1	0.000	1.00	A
B327 2	10	3	2	0.555	.50	A
B327 6	9	2	4	1.799	.30	A
B327 8	14	0	1	2.688	.10	A
B327 9	16	0	0	5.333	.02	B
B327 12	12	3	0	0.200	.70	A
B327 14	15	0	0	4.947	.02	B
B328 2	11	2	0	0.640	.50	A
B328 3	9	3	3	1.799	.30	A
B328 4	14	2	0	1.333	.20	A
B328 5	14	0	0	5.167	.02	B
B328 6	15	0	0	4.947	.02	B
B328 7	13	0	2	1.088	.30	A
B328 8	13	0	0	4.333	.04	B
B328 9	14	1	1	1.333	.20	A
B328 12	10	4	1	0.555	.50	A
B328 15	11	4	2	0.960	.70	A
B328 16	6	5	0	0.777	.50	A
B329 2	14	0	0	5.167	.02	B
B329 5	14	1	0	2.688	.10	A
B329 8	9	6	0	1.799	.30	A
B329 10	10	2	0	0.444	.50	A
B329 12	8	4	3	3.755	.05	A
B329 15	16	0	0	5.333	.02	B
B329 16	13	1	1	1.088	.30	A
B330 3	9	7	0	2.750	.10	A
B330 5	12	3	1	0.000	1.00	A
B330 8	2	1	12	30.421	.00	C
B330 12	11	2	3	0.333	.50	A
B330 15	13	3	0	0.333	.50	A

\*"A" represents progenies with  $P$  values of .05 or higher. "B" and "C" stand for those with  $P$  values below .05. The 10 B progenies appear to be pure breeding greens. The four C progenies are those consisting almost exclusively of albinos.

four green  $F_2$  plants that produced few or no green offspring are excluded, the ratio obtained is 10 homozygous to 34 heterozygous green  $F_2$  plants. The goodness of fit to a two to one ratio is indicated by  $\chi^2$  of 2.23 and  $P$  of .15.

The progenies shown in Table VII fall into three general groups on the basis of the  $\chi^2$  tests as shown in the last column of the table. The  $A$  progenies are those that statistically may be considered satisfactory fits to a single factor hypothesis for green. The  $B$  progenies are the 10 that appear to be true breeding greens. The  $C$  segregations are four in which the  $F_2$  plants apparently lacked the gene for green colour. Totalling the results for the  $A$  progenies gives 376 green, 105 variegated, and 35 white. The fit for a dominant green gene is very good,  $P$  being .30. In addition there is indication of a dominant hypostatic gene for variegated as the fit to a 12 : 3 : 1 ratio of green, variegated, and white is excellent with a  $\chi^2$  of 1.24 and a  $P$  of .5. If such a hypostatic gene is assumed there would have to be the further assumption of a very high mutation rate from variegated to white.

In Table VIII the  $F_3$  segregation is given for selfed  $F_2$  variegated plants from the cross Regal  $\times$  variegated. Five of the 19 progenies had some

TABLE VIII  
THE  $F_3$  SEGREGATION FROM STRIPED  $F_2$  PLANTS FROM THE CROSS  
REGAL (GREEN)  $\times$  STRIPED

$F_2$ plant No.	$F_3$ plants			$F_2$ plant No.*	$F_3$ plants		
	Green	Striped	White		Green	Striped	White
B323 6	0	1	7	B323 10	8	1	5
B323 11	0	0	12	B323 14	8	1	4
B324 13	0	1	11	B323 15	12	0	2
B325 2	0	0	9	B325 11	10	3	2
B325 7	0	0	9	B326 11	13	3	0
B326 2	1	2	13	Totals	52	8	13
B326 5	0	0	14				
B328 14	0	2	7				
B329 11	0	1	15				
B329 13	0	1	4				
B329 14	0	1	15				
B330 4	0	1	15				
B330 11	0	4	12				
Totals	—	14	143				

\* These five plants had 52 green and 21 other progeny. The fit to a 3 : 1 ratio is good,  $\chi^2$  being 1.10 and  $P$  being .5.

green plants and their segregations both separately and collectively showed good fits to a 3 : 1 ratio for green to others ( $P$  being .5). It may be assumed that the five plants are heterozygous for variegation and are genetically like the 34 heterozygous greens listed in Table VII. The results from this group do not support a two factor interpretation. Since striped plants that segregate produce a larger proportion of albino offspring than do segregating green plants, a direct effect of plastid transmission through the egg is indicated.

The cytological study gave the following results. Mesophyll cells of green leaves were found to contain many chloroplastids. Few plastids of any kind were detected in mesophyll cells of white leaves or the white portions of variegated leaves. Many small bodies mostly less than  $2\ \mu$  in diameter were in these cells. The iodine test for starch on white leaves usually gave no starch reaction. A slight trace of starch was found rarely in leaves of albino seedlings. To determine whether mesophyll cells of "white" tissues were well supplied with plastids capable of depositing starch, the cut tips of detached white leaves were immersed in 10% sucrose solution for 24 to 48 hr., then tested for starch and freehand sections examined. Numerous starch grains of sizes similar to those in green plastids were formed. It is concluded therefore that mesophyll cells of white leaves contain many small colourless plastids. Presumably these plastids are colourless derivatives of mutated chloroplastids.

No visible chromosome irregularity was detected in acetocarmine mounts of pollen mother cells of variegated barley. Seven normal-appearing bivalents were observed in many cells. These were similar to the seven bivalents seen in pollen mother cells of Regal barley. Figs. 2 and 3 represent cells of Regal and variegated, respectively, at diakinesis. A few preparations from  $F_1$  plants of green  $\times$  variegated have been examined without any chromosome irregularity being noticed. Fig. 4 shows an  $F_1$  microsporocyte with seven bivalent chromosomes.



FIG. 2. *Regal barley. Diakinesis. Seven bivalents.  $\times 1000$ .*

FIG. 3. *Variegated barley. Diakinesis. Seven bivalents.  $\times 1000$ .*

FIG. 4.  *$F_1$  of *Regal*  $\times$  *variegated* barley. Diakinesis. Seven bivalents.  $\times 1000$ .*

### Discussion

Characters dependent on plastid characters are of special interest to students of gene physiology. Plastids, like genes or chromosomes, are capable of reproducing their kind by division. Proplastids, which also form plastids, may be directly related by descent to pre-existing plastids or proplastids. Although the plastid can reproduce itself it does not have full control over its own constitution. A mutated nuclear gene may deprive some or all plastids of chlorophyll. According to Rhoades (12) the *iojap* gene on chromosome seven in corn induces plastid mutation. Not all the plastids mutate so that some leaf sectors are green. The white plastids even in the presence of the normal 'plus' gene do not mutate back to green. In the variegated barley studied by Imai (5, 6) a somewhat similar explanation was put forward.



One of two hypotheses suggested to account for the results obtained in this investigation assumes that the explanation of the peculiarities of inheritance in our variegated barley resembles that given by Rhoades (12) for the inheritance of the iojap character in corn. On this hypothesis (called Hypothesis I) a single gene mutation was responsible for the origin of the variegated and albino phenotypes. Plastid mutation induced by the mutated gene followed by the segregation of plastids in tissues and transmission through eggs is directly responsible for the occurrence of striped leaves and also for the increasing proportion of albinos in successive generations. The other, which will be known as Hypothesis II, assumes two dominant genes for green, one epistatic to the other with rare mutation in the major gene but a high mutation rate in the other gene in the absence of the first one. As both theories include the presence of a main dominant gene the summarized data from Tables V to VIII may be scrutinized in that regard. The pertinent data are as follows:

Table	Cross	Green	Striped	White	<i>P</i> values for	
					3 : 1	12 : 3 : 1
V	Hannchen × striped	151	27	9	.05	.06
VI	Striped × 3 varieties	255	43	37	.7	.00
VII	Selfed heterozygotes	376	105	35	.3	.50
VIII	Selfed heterozygotes	52	8	13	.5	.00
Totals		834	183	94	.9	.01
Totals for Tables V and VII		527	132	44	.9	.90

This tabulation does not include any  $F_2$  or  $F_3$  data showing a very poor  $P$  value for a fit to a 3 to 1 ratio of green plants to others. The combined data show an excellent fit to a 3 to 1 ratio. It is of interest that of the 66  $F_2$  plants given a progeny test in  $F_3$ , four of the 48 recorded as green (Table VII) had the typical "inbred striped" breeding behaviour and five of the 18 recorded as striped (Table VIII) segregated like typical heterozygous greens. The corrected  $F_2$  classification of the Regal × variegated cross is 10 pure for green ( $WW$ ), 39 heterozygous ( $Ww$ ), and 17 typical for striping ( $ww$ ). These results give a  $P$  of over .5 for a fit to a three to one ratio. The extent of the errors in the classification of  $F_2$  plants is not unusual especially for a character so variable in expression as striping. It seems reasonable that striped plants with a negligible amount of white striping would appear to be green. It is not easy to explain how genetically green plants could appear to be striped unless white plastids were transmitted directly through eggs of a variegated mother or plastid mutation occurred in heterozygous greens.

Now we turn to the results from selfed striped plants and striped × striped crosses. The percentage figures on all the data are brought together in the following tabulation.

Table	Material	Percentages of plants		
		Green	Striped	White
1	19 striped plants	1.2	14.5	84.3
2	9 striped plants	0.0	6.9	93.1
2	17 striped plants	0.0	7.0	93.0
2	30 $\pm$ striped plants	0.0	9.0	90.0
3	2 striped plants	0.2	10.9	88.9
8	13 striped plants	0.0	8.9	91.1
	Unweighted averages	0.2	9.1	90.3

Apart from the very large proportion of albino offspring of selfed variegated plants, it is of interest to note that the proportion of whites was higher in the second generation of inbreeding (Table II) than in the first (Table I).

In considering the above figures the  $F_1$  results should be kept in mind. Summarizing from Table IV we have the following  $F_1$  results:

—	Green	Striped	White
Striped $\times$ green	11	4	7
Green $\times$ striped	41	1	0

The two hypotheses suggested to account for the recorded facts are outlined in some detail below.

#### *Hypothesis I*

Inbred variegated and albino plants are genetically alike as far as nuclear genes are concerned. They differ in that variegated plants have two kinds of plastids each reproducing its kind, while albino plants have only mutant white plastids. The egg cells of variegated plants may have all green, mixed, or all white plastids. In the presence of the mutant gene,  $w$ , mutation is induced in some of the green plastids of eggs or zygotes containing them. Segregation of green and white plastids at successive cell divisions brings about the striped-leaf effect. Since nearly all the  $F_1$  plants were green when the female parent was green but were green, white, or striped when the female parent was striped, it is apparent that the plastids or proplastids present in the egg at the time of fertilization affect the phenotype of the individual that develops from it. Heterozygous individuals may be phenotypically green, white, or striped. Dominance of the gene for green ( $W$ ) is indicated by the close fit to a three green to one of all others in  $F_2$  as well as by the high proportion of green  $F_1$  plants. That the gene  $w$  may induce plastid mutation at a low rate when in the heterozygous condition is indicated by the rare appearance of striped offspring from the green  $\varnothing \times$  variegated male cross. According to Imai (6) there is no plastid transmission through the males in barley. Striped  $F_2$  plants from green  $F_1$ , which produced, when

selfed, green to others in a three to one ratio, also indicate the occasional effectiveness of  $w$  in heterozygous condition in bringing about plastid mutations.

The relatively high proportion of variegated segregates as compared to the number of albinos in  $F_2$  and  $F_3$  progenies from green heterozygotes may be explained on this hypothesis as follows: the eggs giving rise to these individuals receive green plastids in all cases from the green parent. In order to produce a full albino, all of the plastids must mutate to white early in development. The plastid mutation rate is high enough to accomplish this only in about one-quarter of the individuals. In the remainder only a part of the plastids have mutated.

The wide variation in the proportions of albinos from different heads of the same inbred variegated plant (Table III) may be attributed to plastid segregation. Very little green tissue is present in some heads while others have much. In the latter a considerable proportion of egg cells may be equipped with chloroplastids while in the former few or none have chloroplastids.

As mentioned in the introduction there are a number of reports of maternal plastid inheritance. Of these earlier instances the one most resembling the situation described in this paper is that of Imai (5). Our barley differs most strikingly from Imai's in that it produces on selfing about 90% of albino offspring compared to 10% in his.

Apart from plastids there have been few reports of cytoplasmic inheritance. Cytoplasmic transmission of a male-sterility factor was reported by Rhoades (11) and several other accounts have been reviewed recently by Darlington (4).

### *Hypothesis II*

The presence of a second gene is indicated by the data of Tables V and VII where there is a very good fit to a 3 : 1 ratio for the striped and white plants. The pertinent data show 527 green, 132 striped, and 44 white, which gives an excellent fit to a 12 : 3 : 1 ratio,  $P$  being .90. Assuming that the factorial constitution of a normal green plant is  $WWW_1W_1$  and that of a striped plant is  $wwW_1W_1$  the difference having occurred originally through a point mutation of  $W$ , we can arrive at the results of Tables I to IV through mutation in  $W_1$ . It would be necessary to postulate that  $W_1$  is relatively stable in the presence of  $W$  but so unstable in the absence of  $W$  that mutations of  $W_1$  to  $w_1$  are frequent. In that case a self-fertilized  $wwW_1W_1$  embryo might by mutation become  $wwW_1w_1$ , which would still give a striped plant, or even  $www_1w_1$ , which would become a white plant. Repeating the selfing of striped plants would involve some  $wwW_1w_1$  plants, which, with a continued high mutation rate, would be expected to produce a very high proportion of whites.

The case in favour of Theory I is supported by the work of Imai and others. On the other hand, Theory II is not unreasonable and should be kept in mind in the case of further work being done.

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## INVESTIGATIONS OF DECAY IN WESTERN RED CEDAR IN BRITISH COLUMBIA<sup>1</sup>

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### Abstract

Investigations of decay in *Thuja plicata* D. Don. in British Columbia have shown that the major heart-rotting fungi of living western red cedar on the Coast, in decreasing order of importance, are *Poria asiatica* (Pilát) Overh. (brown cubical pocket and butt rot), *Poria albipellucida* Baxter (white ring rot), *Fomes Pini* (Thore) Lloyd (white pitted trunk rot), *Merulius* sp. (brown crumbly butt rot), and *Poria subacida* (Peck) Sacc. (spongy white rot). In the Interior of the Province they are *Poria asiatica*, *P. Weirii* Murr. (yellow ring rot), *Fomes Pini*, *Polyporus balsameus* Peck (brown cubical butt rot), *Merulius* sp., and *Poria subacida*. Other fungi attacking the heartwood of living trees less extensively are *Fomes annosus* (Fr.) Cke., *F. nigrolimitatus* (Romell) Egel., *F. pinicola* (Sw.) Cke., *Armillaria mellea* (Fr.) Quél., *Omphalia campanella* (Fr.) Quél., *Polyporus Schweinitzii* Fr., and *Coniophora cerebella* Pers.

On the areas investigated, loss through decay in stands 50 to 450 years of age never exceeded growth increment. It was indicated that decay in younger stands was of importance. Loss through decay and incidence of infection was appreciably higher in the Interior than on the Coast. No reliable external indications of decay in the standing tree were found.

Deterioration in felled trees was caused by *Poria Weirii*, *P. albipellucida*, *P. subacida*, *Armillaria mellea*, *Omphalia campanella*, *Fomes annosus*, *F. nigrolimitatus*, and *F. pinicola*. *Polyporus cuneatus* (Murr.) Overh., and *Hymenochaete tabacina* (Sow.) Lév. were the major decay organisms of cedar sapwood and slash. *Polyporus abietinus* (Dicks.) Fr., *P. versicolor* (L.) Fr., *P. semipileatus* Peck, *P. hirsutus* (Wulf.) Fr., and *Poria isabellina* (Fr.) Overh. were restricted in distribution or of irregular occurrence.

To assist in the identification of cultures all the major heart-rotting organisms were grown on tannic and gallic acid media. Seventy-seven species of Basidiomycetes were collected on living and dead *Thuja plicata* in British Columbia from 1943 to 1945.

### Introduction

The decays of western red cedar, *Thuja plicata* D. Don., have received little attention in the past. This apparent neglect probably arose because of the well-established reputation of cedar heartwood for high resistance to decay under service conditions, with the resultant lack of interest in the fungi that occurred in living trees. However, the ever-increasing shipments of cedar from British Columbia to distant markets, as lumber, poles, or other products, has aroused interest in the cause of certain discolorations in the heartwood. In response to requests that a study of these discolorations be carried out, an investigation was initiated in 1943.

Western red cedar is one of the most important softwoods of British Columbia. The British Columbia Forest Branch has reported (19) that

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Contribution from the Graduate School, Yale University, New Haven, Conn., and from the Division of Botany and Plant Pathology (No. 868), Science Service, Department of Agriculture, Ottawa. This paper constitutes part of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the Graduate School, Yale University, May, 1945.

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there is a total of 24,597 million bd. ft. of accessible cedar of merchantable size in the province. In total volume this species ranks second only to western hemlock. The average annual cut (9) for the past 10 years amounted to 567 million bd. ft., standing second to that of Douglas fir.

Prior to this investigation little was known of the occurrence and relative importance of the various fungi causing decay of western red cedar. During recent years a number of attempts have been made to determine by cultural methods the identity of the heart-rotting fungi, particularly those causing the brown cubical decays, but these have been abandoned or set aside because of the difficulty in isolating the organisms concerned. On reading the literature on the decays of cedar it is found that only one of the major decays is ascribed to a known fungus, that being the yellow ring rot caused by *Poria Weirii* Murr. Other observational reports were published on the occurrence of *Fomes Pini* (Thore) Lloyd, *Polyporus Schweinitzii* Fr., *Armillaria mellea* (Fr.) Quél., and *Poria subacida* (Peck) Sacc. on cedar, but no data were collected on the relative importance of these wood-rotting organisms. The first phase of this investigation was, therefore, to determine the fungi responsible for the decays and to ascertain the relative importance of each.

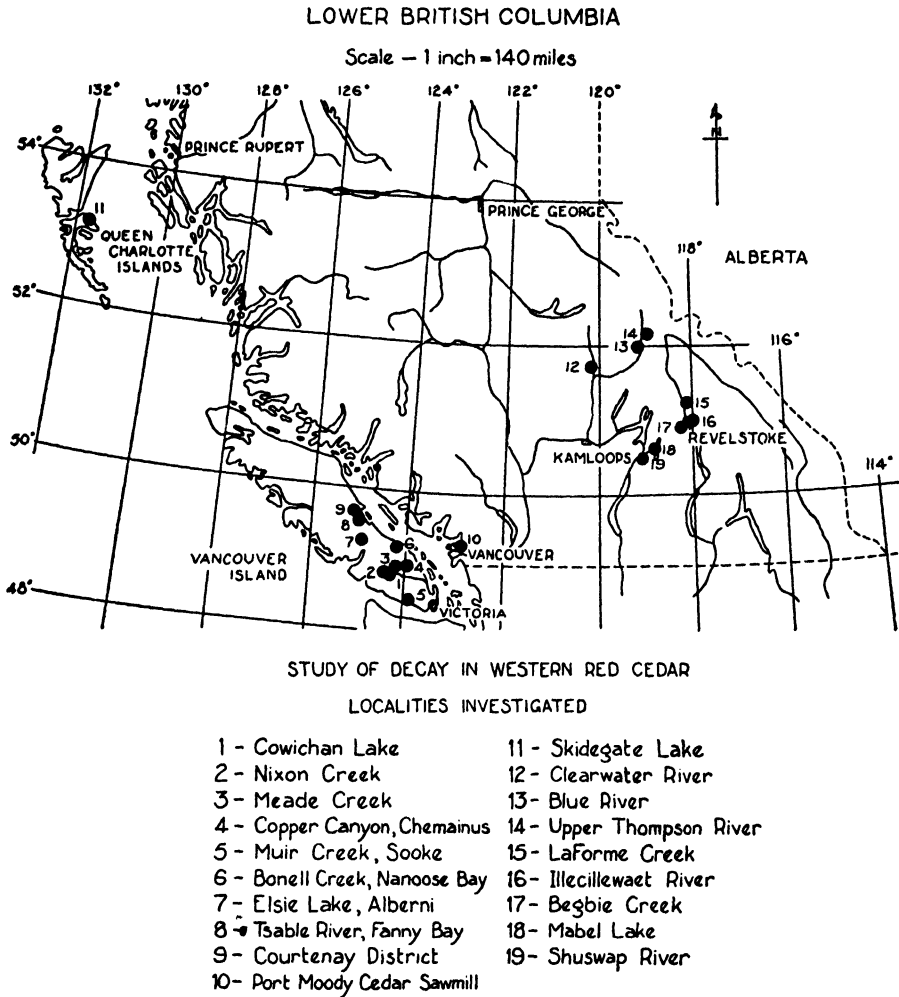
A total of 615 trees was examined in 11 localities on the Coast\*, and 110 trees in eight localities in the Interior (Text-fig. 1).

### Methods of Study

As the first phase of this study was to be an attempt to separate and name the fungi causing decay in western red cedar, and to become familiar with the gross characteristics of the decays, the choice of areas was not the best for collecting data to determine the relative importance of the wood-destroying fungi in relation to age of the host. To obtain as complete a picture of the distribution of the wood-destroying fungi as possible, the localities for study were chosen over a wide range. The governing features in the choice of an area were the numerical abundance of cedar, the freshness of cut, and the accessibility of the stand to laboratory facilities. These requirements were most satisfactorily met in localities where pole-cutting operations were being carried out.

A 100% cruise of all felled western red cedar on the areas under investigation was attempted. Local topography was occasionally so steep or rugged that as low as 80% of the trees were inspected. On every tree measurements were taken of the stump height, stump diameter, and the diameter at critical intervals along the stem. A count of the annual rings was made at stump height and the total age calculated by estimating the number of years it took the tree to grow to stump height. The frequent occurrence of hollow butts made this age count impossible on many trees. In such cases the annual rings were counted as close to the butt as possible and the estimated time the

\* The term "Coast" is used to refer to all areas of British Columbia west of the Coast Range, while "Interior" refers to all areas east of these mountains.



TEXT-FIG. 1. *Localities investigated in the study of decay in western red cedar.*

tree required to grow to this height was added, giving a reliable approximation of the total age.

Complete information was noted in the field on the type of decay, the extent, similarity to other infections in the area, probable entrance point, and the extent, character, and age of any wounds or deformities. Whenever possible a large collection of the decay in incipient and advanced stages was made. The specimens of decay were removed to the laboratory as shortly after collection as possible so that culturing of the organisms could be carried out while the specimens were still fresh.

All trees were plotted on British Columbia Forest Service Form 6503, a form similar to the United States Forest Service Form 558a, and the cubic volumes were measured from a standard stump height of 3 ft. to a 6-in.

merchantable top for all pole timbers, and a 12-in. merchantable top for saw-log timbers. This computation involved the measuring of the area between the outline of the plotted tree and the diameter and height axes with a planimeter, and multiplying the result by a conversion factor. All measurements were made twice, and if the two readings did not check to within 1%, the measurements were repeated. Computations were carried to two decimal places, and the resulting figures were taken to the nearest tenth of a cubic foot. This procedure was repeated for the measurement of the decay column in infected trees.

In all cases where doubt existed concerning the identity of the decay in the field, a specimen was taken to the laboratory for inspection and culturing. In the laboratory all decay specimens giving similar organisms in culture were grouped. Those from which no cultures were obtained, but which closely resembled a typical specimen of known rot, were grouped with the known decay for computing the relative importance of this decay.

### Description of the Decays

For the purpose of this study, the decays were separated into white and brown rots, depending on the reaction of the causal fungus on tannic and gallic acid media (11). In general the organisms causing white rot give a positive reaction on tannic and gallic acid media, staining the media brown, while those causing brown rot give no reaction on these media. Thirteen species of fungi have been found to cause decay of the heartwood of living cedar. Of these, eight cause white rots and five brown rots.

#### WHITE ROTS

Four fungi capable of producing white rots of importance and four of possible significance in localized areas have been found in western red cedar. The major wood-destroying organisms have been identified as *Poria albipellucida* Baxter, *Poria Weirii*, *Fomes Pini*, and *Poria subacida*. The four of secondary importance were identified as *Fomes annosus* (Fr.) Cke., *Armillaria mellea*, *Omphalia campanella* (Fr.) Quél., and *Fomes nigrolimitatus* (Romell) Egel.

#### *White Ring Rot (Poria albipellucida) and Yellow Ring Rot (Poria Weirii)*

The decays caused by *Poria albipellucida* and *Poria Weirii* have been found to be very similar in gross appearance. This similarity is apparently the reason that the decay caused by *P. albipellucida* has been almost unrecognized in the past, all the ring rot of cedar being attributed to *P. Weirii*. That an organism other than *P. Weirii* was known to cause a ring rot in cedar on the Coast was indicated by Eades and Alexander (12) and others.

*P. albipellucida* (3) (Pl. I, Figs. 1 and 2) and *P. Weirii* (21) (Pl. II, Fig. 1) both cause a white to yellow ring rot of living western red cedar. The incipient stage is first manifested by a yellowing in the reddish-brown heartwood, the wood remaining firm. With *P. albipellucida* decay, occasionally a



bluish, marginal discoloration or the appearance of darker, reddish stains at the limits of infection are observed. Later the wood becomes much softened and the discoloration is more evident. In the advanced stage the annual rings separate, the earlywood decaying more readily than the latewood, and the typical, laminated ring rot appears. In this stage the rots caused by the two fungi can be separated. *P. albipellucida* ring rot very commonly shows conspicuous, white, mycelial flecks between the laminae, and the wood surface shows minute, elongate, fine pits (Pl. I, Fig. 3). The late advanced stage is a typical ring rot, rarely fibrous in nature (Pl. I, Figs. 6 and 7). On the other hand, *P. Weirii* ring rot sometimes shows small patches or a thin web of setal hyphae, dark brown in colour, between the laminae, and the wood shows long striations, or thin, closely-packed pockets, which, before the complete separation of the laminae, contain white material, probably cellulose products (Pl. II, Figs. 2 and 3). This striation of the laminae gives the advanced rot a fibrous texture. The pole-cutting operators in the Interior refer to *P. Weirii* rot as 'paper rot', as the advanced stage is a mass of thin, flexible laminae (Pl. II, Fig. 4). On the Coast this term has not been applied to *P. albipellucida* decay, as the final stage is coarsely laminated or a crumbly, amorphous mass. Insects, particularly ants and termites, seem to favour this latter rot and are commonly found associated with it.

Present evidence based on identification of decay organisms by cultural methods or by direct examination of the rots (Table I) suggests that, as an important heart-rotting organism of cedar, *P. albipellucida* is limited to the Coast, while the major damage by *P. Weirii* is in the Interior. Although both

PLATE I. FIGS. 1 to 7. *Poria albipellucida* on western red cedar.

FIG. 1. Sporophore on slash.  $\times 1$ .

FIG. 2. Sporophore on a railroad tie.  $\times 1$ .

FIG. 3. Tangential view of heartwood showing the advanced stage of white laminate rot with mycelial flecks.  $\times \frac{1}{2}$ .

FIG. 4. Cross section through the butt of a freshly cut pole showing the incipient stage of white laminate rot.

FIG. 5. The same pole after seasoning showing the formation of ring shakes in the heartwood.

FIG. 6. Longitudinal section of cedar showing the advanced stage of white laminate rot.  $\times \frac{1}{2}$ .

FIG. 7. Cross section through a log showing the incipient and advanced stages of white laminate decay.

PLATE II. FIGS. 1 to 4. *Poria Weirii* on western red cedar.

FIG. 1. Sporophore showing stratification, on the lower side of a log.  $\times \frac{1}{3}$ .

FIG. 2. Tangential view of heartwood showing the advanced stage of yellow ring rot.  $\times \frac{1}{2}$ .

FIG. 3. Longitudinal section of a young cedar showing the heartwood completely rotted, and a mat of setal hyphae over the surface of the exposed infected wood.  $\times \frac{1}{2}$ .

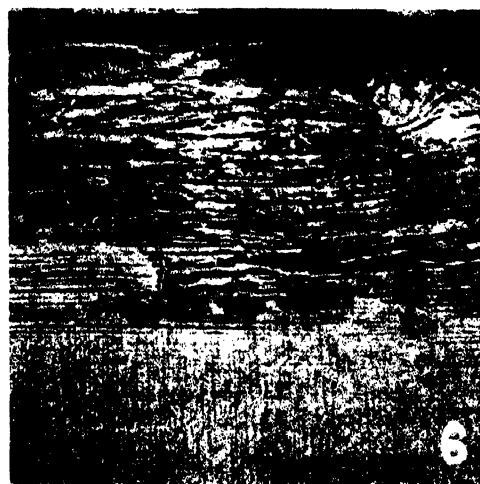
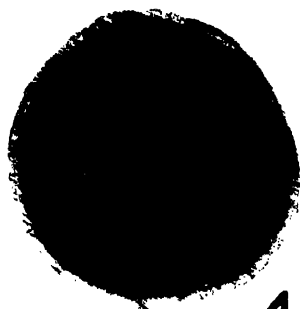
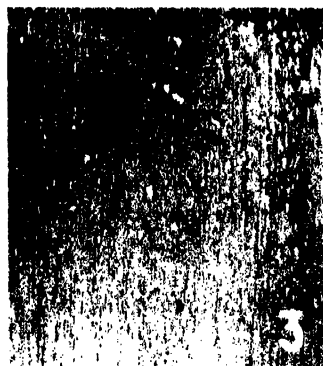
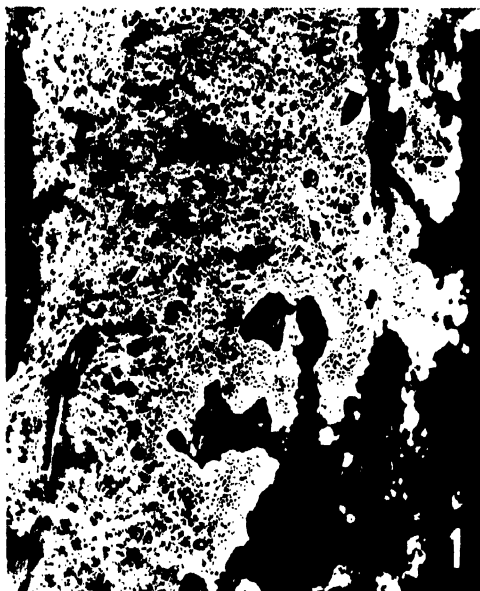
FIG. 4. Longitudinal section of a log showing the advanced stage of yellow ring rot.

FIGS. 5 to 7. *Poria subacida* on western red cedar.

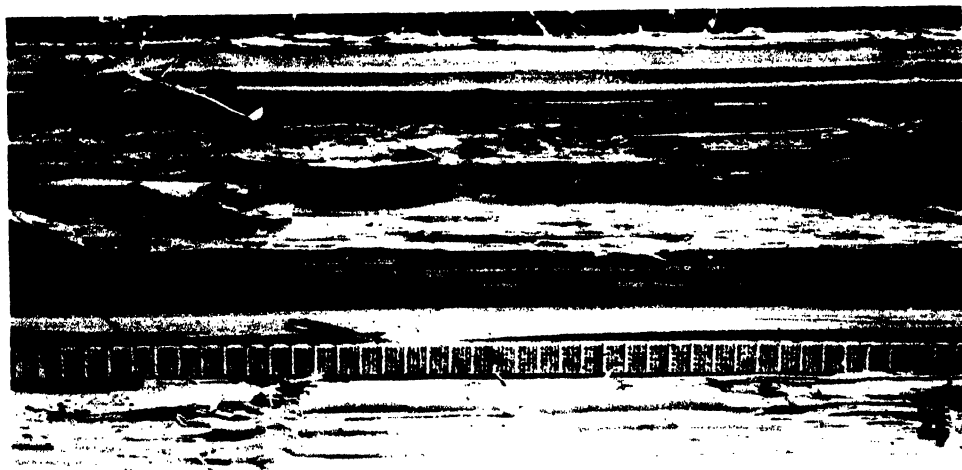
FIG. 5. An annual sporophore growing on the side of a living tree.

FIG. 6. Tangential view of heartwood showing the advanced stage of white spongy rot with white rhizomorph-like strands between the laminae.  $\times \frac{1}{2}$ .

FIG. 7. Cross section through a log showing the incipient and advanced stages of white spongy rot.









these organisms are present in both regions, it is doubtful whether *P. albipellucida* attacks living trees in the Interior, while *P. Weirii* attacks such trees only to a limited extent on the Coast, occasionally causing damage in young growth (18) and mature trees. The reason for the regional activity of these organisms is obscure.

TABLE I

REGIONAL AND HOST AGE-CLASS DISTRIBUTION OF ISOLATIONS AND AUTHENTIC RECORDS OF *Poria Weirii* AND *Poria albipellucida*

Age class of infected trees	Number of isolations and authentic records			
	<i>Poria Weirii</i>		<i>Poria albipellucida</i>	
Years	Coast	Interior	Coast	Interior
50	1	2	2	—
100	—	—	2	—
150	—	3	11	—
200	—	10	32	1
250	—	17	16	—
300	1	3	—	—
350	—	1	5	—
400	—	—	3	—
Total	2	36	71	1

#### *White Pocket Rot (Fomes Pini)*

White pocket rot or conk rot, caused by *Fomes Pini* (25), was found to be of some importance in all cedar stands (Pl. III, Figs. 1 and 2). The description of the decay as given by Baxter (5), Boyce (8), and Hubert (15), for other hosts applies for the most part to cedar.

The incipient stage is usually not easily distinguished beyond the advanced rot column, occasionally appearing as a slight paling of the heartwood. On the lateral margins of the advanced decay, however, the incipient rot is frequently associated with a narrow, bluish to reddish-brown line or band (Pl. III, Fig. 3). Occasionally in basal infections the decay did not appear typical of *F. Pini*, but was a clearly-marked band of yellow discoloration containing only fine pits and was commonly accompanied by brown zone lines.

#### *Spongy White Rot (Poria subacida)*

A spongy white rot or stringy butt rot of cedar is caused by *Poria subacida* (Pl. II, Fig. 5). This decay is referred to extensively in literature as a spongy rot of *Thuja occidentalis* Linn. and a feather rot of *Abies balsameus* Mill. in the forests of Northeastern America (2, 17, 23, 24).

The incipient stage is usually limited to a thin margin about the advanced decay, where the wood becomes more deeply coloured. The advanced decay, while somewhat laminated in appearance, is readily distinguished from the ring rots of red cedar by the formation of irregularly-shaped pockets, which may coalesce to form a continuous mass of white fibres in the earlywood.

Black flecks sometimes appear in these white fibres. The laminae, on separation, frequently show patches or sheets of white to cream mycelium or rhizomorph-like strands (Pl. II, Fig. 6). In the final stages of decomposition the wood is a feathery to spongy mass, commonly containing numerous secondary decay fungi (Pl. II, Fig. 7). The great majority of infections take place through basal wounds or injured roots.

### Minor White Rots

The spongy butt rot of living western red cedar caused by *Fomes annosus* (4) has been found during the course of this study on young trees 35 to 50 years of age. The fungus is apparently of little importance as a wood-rotting organism of mature cedar, but may prove of some interest in young stands. Day\* reports that this organism has been observed to cause a butt rot of cedar in a number of places in England.

*Armillaria mellea* (25), the honey mushroom, causes a root and butt rot of cedar of some importance in localized areas. In the University of British Columbia forest, Vancouver, a number of young cedars were reported by Dr. J. E. Bier to have been attacked and girdled by this fungus. In the Blue River area (Text-fig. 1, Locality 13) a number of trees of about 200 years of age were found with the butts completely rotted into a yellow-brown, spongy mass, or with one to several of the roots and a small portion of the butts in a similar condition. *A. mellea* was fruiting abundantly on the decayed wood of these trees, occasionally growing from the infected tree one to four feet above the ground. Although *A. mellea* is capable of causing extensive damage in a restricted area it is, in general, unimportant on cedar.

*Omphalia campanella* (13, 20), the little bell *Omphalia*, reported occurring on decayed coniferous logs and stumps, may prove of some interest as a fungus possibly causing decay of living trees. This minute mushroom, the cap 0.25 to 0.75 in. broad, and stipe 0.5 to 1.5 in. long, produces 'aerial rhizomorphs' in culture and in decay pockets (Pl. III, Figs. 4 and 5). The fungus has been

\* Correspondence dated May 2, 1944.

FIGS. 1 to 3. *Fomes Pini* on western red cedar.

FIG. 1. Longitudinal section showing the advanced stage of white pitted trunk rot and a sporophore of the causal fungus growing from a branch stub.  $\times \frac{1}{2}$ .

FIG. 2. Sporophores of the white pitted trunk rot fungus on the end of an infected log.  $\times \frac{3}{4}$ .

FIG. 3. Longitudinal section of heartwood showing the incipient stage with discoloration, and the advanced stage, of white pitted trunk rot.

FIGS. 4 and 5. *Omphalia campanella* on western red cedar.

FIG. 4. Sporophores growing from decayed heartwood.  $\times \frac{1}{6}$ .

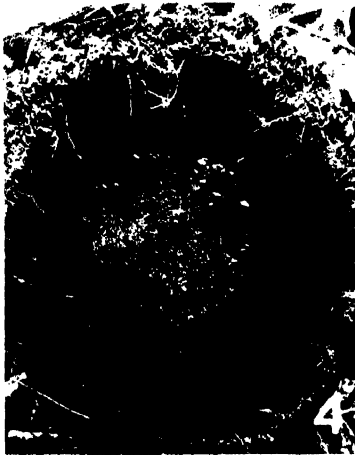
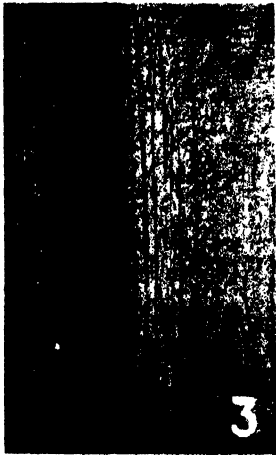
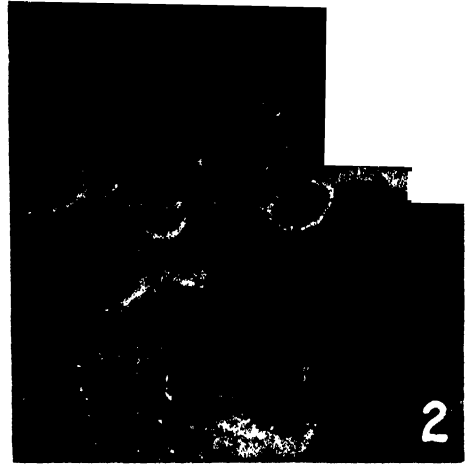
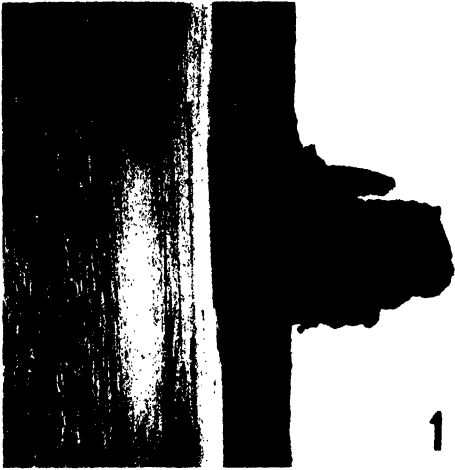
FIG. 5. Longitudinal section of a living tree showing the black 'aerial rhizomorphs' of the fungus in a pocket of brown cubical rot caused by *Poria asiatica*.  $\times \frac{1}{3}$ .

FIGS. 6 and 7. *Polyporus balsameus* on western red cedar.

FIG. 6. Sporophores growing from brown cubical butt rot.  $\times \frac{1}{4}$ .

FIG. 7. Cross section through the butt of a tree showing piped or tubular rot caused by the fungus.

FIG. 8. *Polyporus Schweinitzii* sporophores on the root of a living western red cedar.







found associated with the decays of the white rot fungi, *Poria albipellucida*, *P. Weirii*, and *Fomes Pini*, and the brown cubical rot fungus, *Poria asiatica*. Whenever the fungus has been isolated from the incipient decay associated with a brown cubical rot, invariably 'aerial rhizomorphs' have been found scattered or densely packed throughout the brown cubes. Where this fungus was found with the white rots, no 'aerial rhizomorphs' have been found, but the decay was more yellow-brown than the normal white rot and contained fine, brown zone lines. It seems probable that this fungus is a secondary wood-destroying organism only, growing in association with one of the primary rot fungi. It has been cultured on several occasions from as high as 15 feet up the heartwood of a living tree, however, in pockets with brown cubical rot, and from the upper limits of the incipient decay of *P. Weirii* and other white rots. After authentic cultures of this fungus had been obtained from rot in cedar heartwood and also from associated fruit bodies, similar cultures isolated from black spruce and balsam fir pulpwood bolts in the East, and Douglas fir and living Sitka spruce in the West, were identified as this species. Further investigations on the activity of this fungus are required.

*Fomes nigrolimitatus*, causing a white pocket rot, has been found by R. E. Foster on the Queen Charlotte Islands and on Vancouver Island in living cedar. This fungus has not previously been reported as occurring in living trees. Hubert (15, p. 381) mentions dead material of western red cedar as one of the most common hosts of this fungus.

#### BROWN ROTS

During the course of this study six fungi have been found associated with brown cubical rots of living western red cedar. Two of these, *Poria asiatica* (Pilát) Overh. and *Polyporus balsameus* Peck, are of considerable importance. One, tentatively identified as a species of *Merulius*, is probably of some consequence and three others, *Fomes pinicola* (Sw.) Cke., *Polyporus Schweinitzii*, and *Coniophora cerebella* Pers., occur infrequently.

##### *Brown Cubical Pocket and Butt Rot (Poria asiatica)*

The decay caused by *Poria asiatica* (22, 23), known as "black rot" throughout the Interior, is responsible for the greatest loss in living western red cedar (Pl. IV, Fig. 1). *P. asiatica* causes a brown cubical butt rot and brown cubical pocket trunk rot of living cedar. The incipient stage is easily distinguished from uninfected wood, and extends several feet above the advanced decay column. In this early stage the wood becomes straw-coloured to pale yellow-brown, softened, 'dead' in appearance, and presents a marked contrast to the normal, reddish-brown heartwood (Pl. IV, Fig. 2). In the advanced stage the infected wood turns light brown to brown, becomes brittle, and breaks along shrinkage cracks to form small cubes, the surfaces of which are frequently covered with felt-like mats of white mycelium (Pl. IV, Fig. 5).

In the late stage of decay the type and size of the rot column varies considerably. Frequently a piped or tubular rot was found on the stump, or a

solid mass of decay that did not always appear to be a more advanced stage of the piped condition. Higher in the trunk these two types of basal decay either developed into typical pocket rot or remained an unbroken column of decay. It was found that where the continuous column of decayed wood was present, the infection was usually limited to the basal 4 to 15 feet, while in trees where the pocket rot was present the infection spread from 6 to 80 or more feet up the bole of the tree, growing out the branches in severe infections (Pl. IV, Figs. 4, 7, 8, and 9). In the latter type of decay, pocket rot, the pockets were irregular in shape, 0.5 to 6 in. wide, and 1 in. to 2 ft. in length, separated at times by one to several feet of discoloured wood, or growing together to form a large irregular pocket of decay (Pl. IV, Fig. 5). Variations in the size and type of pocket were very noticeable, and were probably caused by certain inherent properties of the cedar wood hindering, or otherwise affecting, the growth of the attacking fungus. One infrequent type of decay showed pockets varying from minute to 0.5 by 3 in. in size, appearing in a discoloured, infected core that was decidedly harder than normal cedar wood (Pl. IV, Fig. 3).

*P. asiatica* was commonly found in association with one of the major white-rot organisms. In such cases both rots involved were usually separable. Where *P. asiatica* was growing with *P. albipellucida* or *P. Weirii*, the former fungus usually grew through the wood already decayed by the white-rot fungus, and on into the sound wood. Where *P. asiatica* and *Fomes Pini* were growing in the same tree, however, the two fungi grew together commonly with no clear line of demarcation between them, resembling in many respects the condition reported by Boyce in his study of dry rot in incense cedar (6).

#### *Brown Cubical Butt Rot (Polyporus balsameus)*

As *Polyporus balsameus* was responsible for but few of the brown cubical rot infections studied, being found only on trees in the North Thompson and Revelstoke regions in the Interior, only a tentative description of the decay can be presented at this time (Pl. III, Fig. 6). In the incipient stage the decay caused by this organism is a straw to yellow-brown discoloration of the heartwood. The advanced stage is light brown to brown in colour, breaking into fairly large cubes, or into a split crumbly mass, commonly covered with a

#### *Poria asiatica* on western red cedar.

FIG. 1. Sporophores on the end of a log.  $\times \frac{1}{8}$ .

FIG. 2. Longitudinal section of a tree showing the light heartwood with incipient decay and brown cubical rot caused by the fungus.  $\times \frac{1}{8}$ .

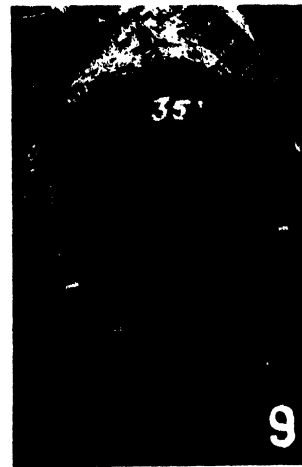
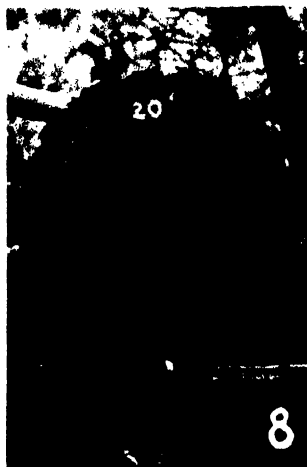
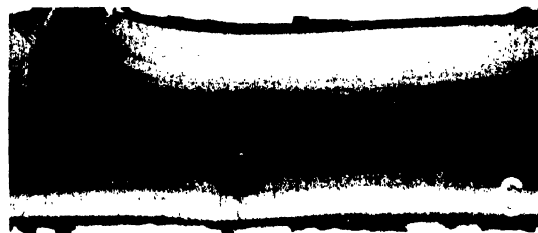
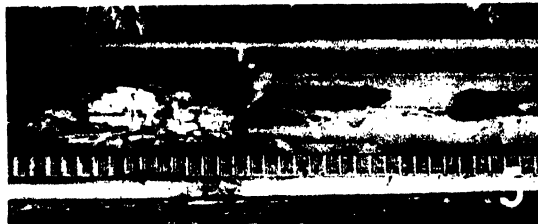
FIG. 3. Longitudinal section showing small pockets of decay caused by the fungus.  $\times \frac{1}{2}$ .

FIG. 4. Cross section 30 feet above the butt of an old tree showing pocket or tubular rot caused by the fungus.

FIG. 5. Longitudinal section of a tree showing pocket rot caused by the fungus.

FIG. 6. Longitudinal section of a small oppressed tree showing the heartwood completely decayed by the fungus.  $\times \frac{1}{2}$ .

FIGS. 7 to 9. Brown cubical pocket rot in a pole tree. Fig. 7, 3 feet from ground-level; Fig. 8, 20 feet from ground-level; Fig. 9, 35 feet from ground-level.





thin web of white mycelium. In three of the infected trees, the infection courts were either small basal wounds or injured roots, and in each of these cases the rot in the end of the butt log appeared typical of this fungus (14, 17), a large, brown cubical rot. In the other three infected trees, where the infection courts were large basal wounds, the rot appeared as a piped or tubular decay in the basal end (Pl. III, Fig. 7). Higher in the trunk the decays arising from both types of infections were very similar in all respects.

Although this fungus was rarely obtained from decay, it seems possible that it is more frequent than these cultures would indicate. If the organism is a butt rot in its usual manifestation, then it is quite possible that a number of the butt rot infections inspected were caused by this fungus, but the large number of secondary organisms present in these infections makes it impossible in many instances to determine this by cultural methods. A further study of fresh specimens may indicate the relative importance of *P. balsameus* as a brown cubical rot of cedar.

#### *Brown Crumbly Butt Rot (Merulius)*

A fungus found by Dr. M. K. Nobles\* to be very similar in growth habits in culture to a species of *Merulius* has occurred in sufficient frequency to warrant attention as a butt-rotting organism of cedar. The incipient stage was usually not clearly indicated, but on occasion a yellow-brown discoloration was found. In the advanced stage considerable variation was evident, but the typical form appeared as a crumbly, light-brown to brown decay, breaking on shrinkage cracks to form large cubes or segments. While most infections resulted in typical butt rots, this fungus was also found entering scars and producing narrow, short, crescent-shaped rot columns in which the brown cubes were usually small and very brittle.

#### *Minor Brown Cubical Rots*

*Fomes pinicola*, the red belt fungus, has been isolated from a light-brown cubical trunk rot of living cedar during the course of this study. One collection and one observation of the sporophores on living trees have been made at Vancouver, B.C. On the Coast *F. pinicola* undoubtedly does occur as a decay of living western red cedar but is of minor importance.

*Polyporus Schweinitzii*, the velvet-top fungus, has long been considered as a possible cause of the brown cubical butt rot of cedar, most of which has been found to be the result of attack by *Poria asiatica*. One culture of this organism from cedar at Vancouver is mentioned by Childs (10), but this culture cannot be traced at present. The fruiting bodies of *P. Schweinitzii* were found on cedar only once in British Columbia, these being attached to the root of a tree 120 years of age (Pl. III, Fig. 8). Although this fungus attacks western red cedar, as reported by Weir (25), it will likely be found to be only of minor importance.

*Coniophora cerebella*, cultured from decay during the course of this investigation, apparently is capable of causing decay in living western red cedar.

\* Correspondence dated 1943.

Cartwright\* reports that the only serious cases of decay in cedar timbers in England have been caused by this fungus. The presence of *C. cerebella* in living trees is, therefore, of considerable interest.

### Entrance of the Decays

Basal wounds and fire scars are the most important avenues for the entrance of decays into living western red cedar. In all localities investigated these injuries were the entrance point of over 90% of the infections. Other points of infection worthy of note, however, are 'stag-head' tops, branch stubs, 'dry-side', and injured roots.

In certain of the dryer areas on the Coast, fire scars were of considerable importance in exposing heartwood to infection, while basal wounds caused by other mechanical decortivating agencies were very frequent generally. In nearly all the localities inspected, but particularly the moister regions of the Interior, basal wounds of doubtful origin were found. These wounds appeared externally as depressed areas in the bark (Pl. V, Fig. 2), limited to a small fraction of the circumference to cases of almost complete girdling. On examination it was found that the cambium had been killed beneath the bark. No correlation was found with any natural causative factor.

Although nearly all scars inspected showed indications of decay, the ability of cedar to heal wounds is very great. A number of cases were observed where the cambium had been killed for over 50% of the circumference, and complete healing had taken place in a relatively short time. In many of these cases excluded hearts were found. Here the callous tissue, rather than growing over the scarred heart, had grown to exclude the heart as illustrated in Pl. V, Figs. 3 and 4, leaving an ever-present entrance for infection. The well-known infolded or fluted base of cedars appears to be chiefly the result of numerous healed basal wounds (Pl. V, Fig. 1). It was observed that all trees inspected, the stumps of which showed no scars were present at any time throughout the life of the trees, were somewhat 'swell-butted', but showed none of the deep infolding or fluting.

\* Correspondence dated May 4, 1944.

FIGS. 1 to 4. Entrance of decay in western red cedar.

FIG. 1. Cross section of part of the butt of a tree showing the bark seam formed in healing a wound infected with *Poria albipellucida*.

FIG. 2. Cross section of three basal wounds caused by an unknown physical or mechanical agency. Three small sections of the cambium are living.

FIG. 3. Illustration of a cross section of a tree at stump height showing how the heart may be excluded laterally during the healing of an extensive wound.

FIG. 4. Illustration of a cross section of a tree at stump height showing how the heart may be excluded centrally during the healing of two or more extensive wounds.

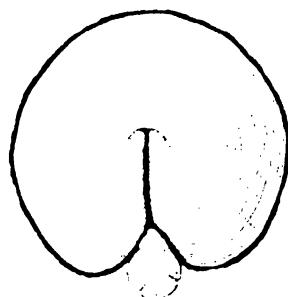
FIG. 5. *Poria albipellucida* on malt agar at the end of six weeks.

FIGS. 6 to 8. *Merulius* sp. in culture.

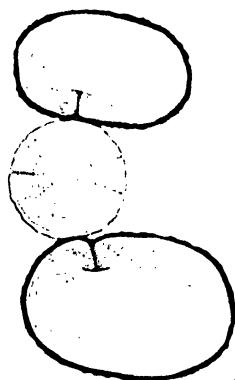
FIG. 6. *Merulius* "A" on potato-dextrose agar at the end of six weeks.

FIG. 7. *Merulius* "A" on gallic acid medium at the end of one week.

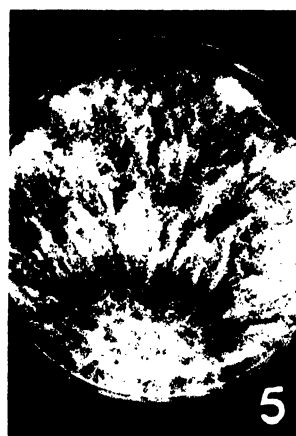
FIG. 8. *Merulius* "B" on gallic acid medium at the end of one week.



3



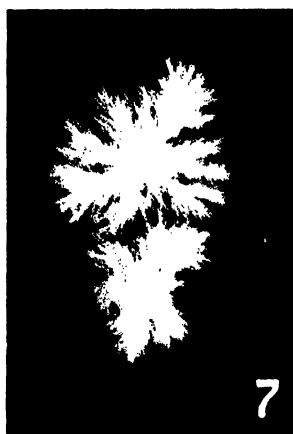
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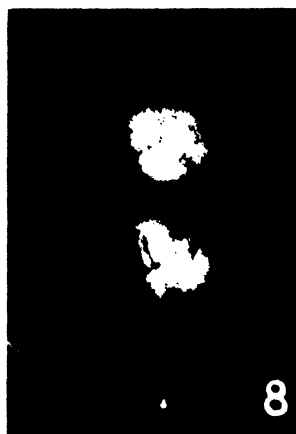
5



6



7



8





'Stag-head' tops, or dead tops, of cedar are a well-known feature associated with this species in all regions, and notably numerous in certain localities. The possible causes of the dead tops have aroused much interest. It would appear that they arise chiefly from some physiological die-back, no pathogens of likely importance having been found associated with the condition. The results of this study to date indicate that dead tops are not an important entrance for decay, and the number of heartwood infections resulting from these tops is too few to consider the defect as a definite indication of decay.

Branch stubs, a common infection court for trunk rots in many species, rarely provide entrance for decay organisms in cedar. A small proportion of the total infections do occur through this avenue, however.

'Dry-side', a defect responsible for the complete cull of a pole tree, is the result of the death of the cambium in a narrow to wide strip down the side of the tree. The cause of 'dry-side' appears to be severe bruising of the cambium tissues by mechanical agencies, such as grazing by a falling tree. Other non-pathogenic factors may be responsible for some of the damage. Except in severe cases the bark usually remains intact over the dead areas and the defect is not diagnosed until the tree is felled. As an avenue for the entrance of decay 'dry-side' was not found to be of importance.

Basal infections through injured roots or means other than wounds were frequent enough, especially in young stands, to warrant further attention than has been given to them in this study. In trees younger than 50 years of age, the well-known root and butt rots, caused by *Poria subacida*, *Armillaria mellea*, *Fomes annosus*, and *Poria Weirii*, have been found to cause appreciable losses in restricted areas, apparently entering through injured roots.

### Relative Importance of the Decays

The study of decay in living western red cedar was complicated by two difficulties: first, in many of the infected areas there were two or more organisms, and it was very difficult in most of these cases to separate clearly the damage caused by each fungus; and second, while the study of decay was in progress, only two of the decays could be attributed to known fungi by gross characteristics. It was found necessary and convenient to study the relative importance of the fungi causing decay by grouping them as in Table II.

The incidence of decay in living trees is illustrated in Text-fig. 2. While this graph is fairly reliable for the south-central portion of Vancouver Island, it probably illustrates an incidence too low for the northern Coast and western portions of Vancouver Island. While the age-class distribution for the Interior was poor, the results would indicate a greater incidence of decay in the younger age classes than is found on the Coast.

The relative importance of the fungi causing these infections is graphically illustrated in Text-fig. 3. For the purpose of this comparison, all the brown cubical rots not clearly distinguished from that caused by *Poria asiatica* were

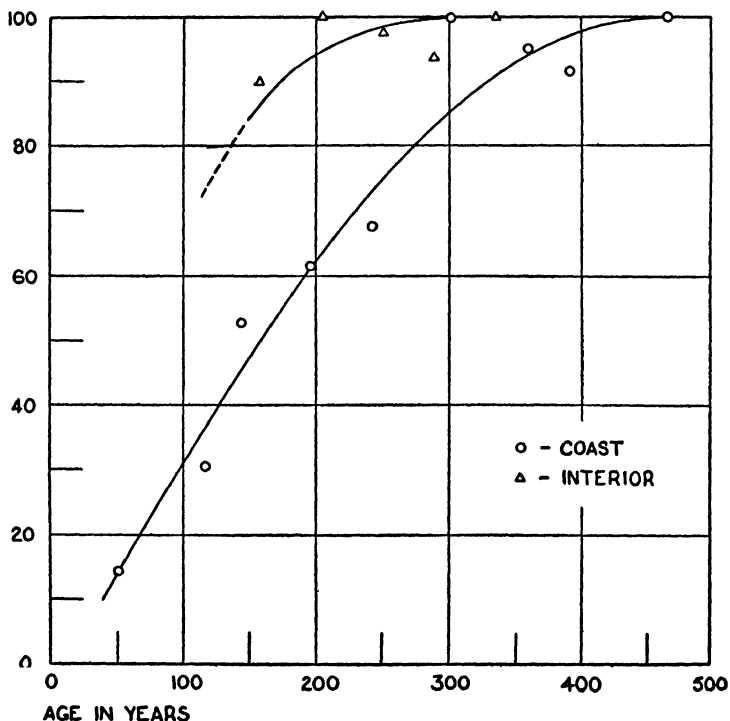
TABLE II

THE NUMBER OF TREES INFECTED BY THE PRINCIPAL WOOD-DESTROYING ORGANISMS\*

Wood-decaying organism	Interior poles		Coast poles		Coast saw-logs		Total	
	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections
<i>Poria Weirii</i>	40	27	0	0	2	0	42	27
<i>Poria asiatica</i>	72	36	79	25	74	37	225	98
<i>Poria albipellucida</i>	1	0	63	21	126	35	190	56
<i>Fomes Pini</i>	16	9	11	4	3	2	30	15
Mixed infections:								
<i>Poria Weirii</i> and <i>Poria asiatica</i>	25	—	0	—	0	—	25	—
<i>Poria albipellucida</i> and <i>Poria asiatica</i>	0	—	21	—	35	—	56	—
<i>Fomes Pini</i> and <i>Poria asiatica</i>	8	—	4	—	2	—	14	—
Inseparable mixtures	5	—	7	—	10	—	22	—

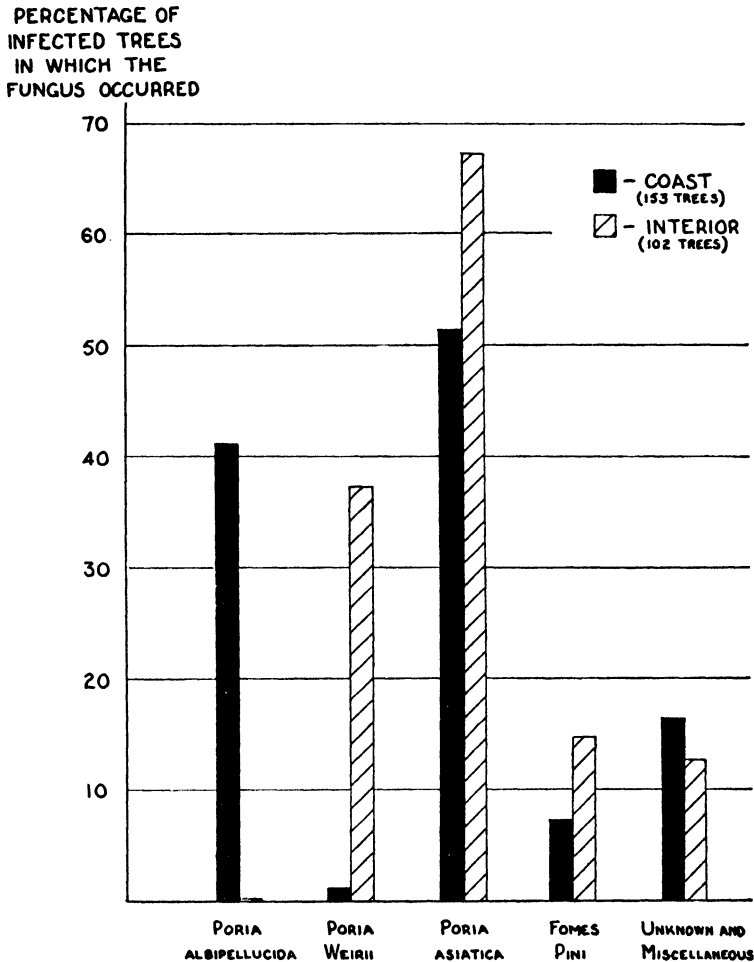
\* One hundred and ten trees were examined in the Interior and 615 trees on the Coast.

PERCENTAGE OF TREES INFECTED



TEXT-FIG. 2. Relation of the occurrence of decay to age in western red cedar.

grouped together under this specific name. *Fomes annosus*, *Poria subacida*, and others were placed under "Unknown and Miscellaneous," as their occurrence was too irregular or unimportant to warrant further attention. It will

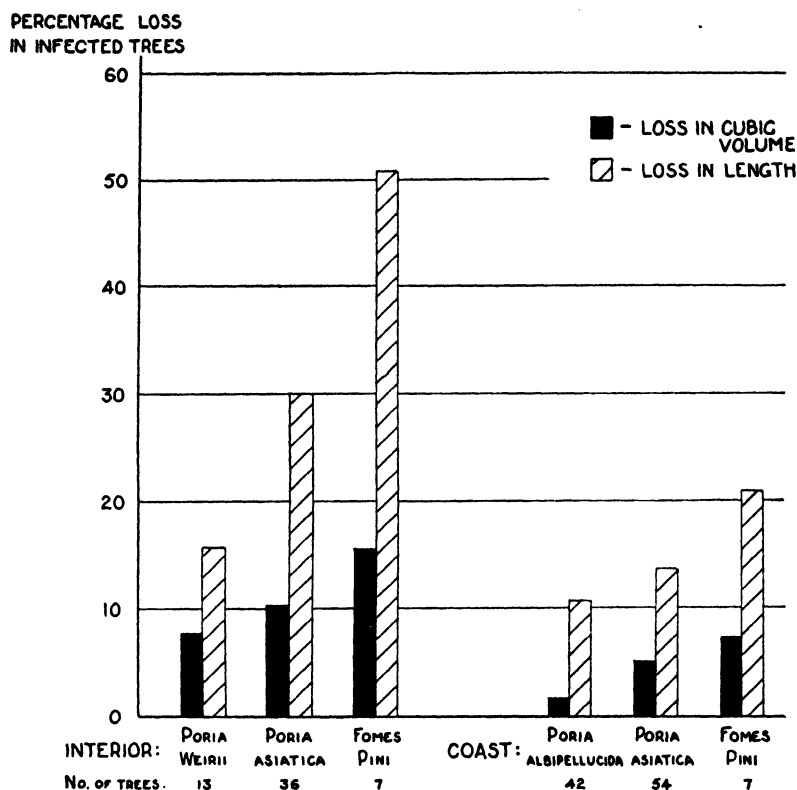


TEXT-FIG. 3. Relative importance of the wood-destroying fungi expressed as a percentage of the total number of infections in diseased trees.

be noted that, on adding together the percentage of infections that occurred, the total exceeds 100% for both regions. The reason for this is the number of mixed infections that occurred, and whenever the two or more attacking fungi could be identified, all species involved were given credit for one infection. The infections by causative fungi are outlined in Table II.

While considering the figures presented, it would seem advisable to inspect the relative losses brought about by the fungi in question. In preparing Text-fig. 4, only trees infected by one organism were considered, and the percentage presented is the average loss in these single-infection trees.

Combining the information presented in Text-fig. 3 with that of Text-fig. 4, it is evident that *Poria asiatica* brown cubical decay is the most destructive on cedar throughout all regions, both in total volume of wood destroyed and



TEXT-FIG. 4. Percentage loss in cubic foot volume and length in infected trees of merchantable pole size by the principal wood-destroying organisms.

in total lineal loss to the pole-cutter. Of the infected trees studied, nearly 70% were attacked by this fungus in the Interior and over 50% on the Coast (Text-fig. 3). The loss in the cedar of the Interior is somewhat greater than on the Coast, 30% of the length and 10% of the cubic volume of infected, pole-sized trees being lost in the former region, and approximately 15% of the length and 5% of the cubic volume of infected trees being destroyed in the latter region (Text-fig. 4). In several cases *Poria asiatica* has been found attacking small, oppressed cedars from 20 to 50 years of age. In such cases the entire heart of the tree was commonly destroyed (Pl. IV, Fig. 6).

*Fomes Pini* decay caused considerably more loss than the brown cubical rots (Text-fig. 4), although the incidence of this fungus was low (Text-fig. 3). Of the infected pole timbers studied in the Interior, only 15% were attacked by *F. Pini*. In these infected trees 15% of the cubic volume and 50% of the length of merchantable pole-sized timbers was destroyed. In the Coast

forests, the incidence was not as great, 7.5% of the total infections being initiated by this fungus. The loss was even proportionally lower, 7% of the cubic volume, and 21% of the length of infected, merchantable pole timbers being affected.

The small loss both in length and cubic volume of poles infected by *Poria albipellucida* and *Poria Weirii* indicates that these are essentially butt-rotting organisms, but the high degree of incidence of *P. albipellucida* on the Coast and *P. Weirii* in the Interior is sufficient indication of their importance. *P. albipellucida*, responsible for over 40% of the infections on the Coast (Text-fig. 3), decays less than 2% of the cubic volume, and causes a loss slightly over 10% of the length of infected pole-sized timbers (Text-fig. 4). *P. Weirii* infection in the Interior, although comparatively less frequent than *P. albipellucida* on the Coast, causes a greater loss, approximately 8% of the cubic volume and 18% of the length of infected timbers.

### Indications of Decay

No reliable method for predicting the amount of decay in standing western red cedar has been found. The almost complete absence of conks on this species makes the problem of estimating decay very difficult.

As previously discussed, basal wounds are the most important points of entrance for decay fungi. These injuries, however, are of very doubtful value in diagnosing the extent of decay in the heartwood. Although the entire butt may appear decayed, the decay column may have grown up only a short distance past the wounds, while in other cases an apparently sound wound may have provided entrance for a fungus that has completely destroyed the upper merchantable portion of the tree. The number, size, and condition of the basal wounds were thus found to be almost valueless in predicting the amount of decay in a standing tree.

'Stag-head' or dead top and 'dry-side' are not indicative of internal decay. In certain regions where cedar is very decadent trees with these defects offer more possibility of extensive trunk decay. Even in such areas, however, unless a considerable portion of the crown were dead or the 'dry-side' very extensive, it was not common to find decay entering these defects.

Rotten knots and conks, if these can be seen before felling the tree, indicate complete loss of the merchantable bole, unless, as in the case of *Poria Weirii* and *P. albipellucida*, the conks are those of butt-rotting organisms. It is in but a small proportion of the cases where the bole is unmerchantable, however, that either of these indications is present.

In felled trees the discoloration of the wood, particularly in association with the decays caused by *P. albipellucida* and *P. asiatica*, was found to be a fairly good indication of the presence of the fungus, but not of the extent of the decay. After long-butting a pole to remove the decay it was frequently observed that a light core of small diameter, up to 8 in. or more across, was left in the base of the pole. This light brown heart was found to be the

incipient stage of either *P. asiatica* or *P. albipellucida*. In freshly cut logs this is very easily detected in cases of attack by the first fungus, the light heart appearing 'dead' in contrast to the normal brown wood. *P. albipellucida* is not so conspicuous in the incipient stage of decay in a freshly cut log, but as drying takes place it is readily seen, the wood cracking in concentric circles (Pl. I, Figs. 4 and 5).

Up to the present no definite correlation has been found between the diameter of the rot column and its length, or between the species of attacking fungus and the length of the rot column.

### Relation of Decay to Age and Vigour of Trees

The normal procedure of plotting periodic growth increment against the decay loss for the same period to determine the pathological rotation of the species, or to find the age at which decay loss exceeds growth increment, showed that at no time during the 450 years of growth covered by this study, did decay increment equal or exceed growth. The limited data upon which these computations were made may have distorted the true results, but it seems probable that such an observation on western red cedar may be true for the age period studied.

In trees of vigorous growth, 35 to 50 years of age, many of the major decay organisms of mature trees were found to cause severe damage. For example, *Poria albipellucida* and *P. Weirii* were found to infect all the heartwood in several cases, a condition not found in older trees. In one area on Vancouver Island where 50 trees averaging 50 years of age were inspected, two out of a total of seven infections were by *Fomes Pini* and both these were through basal wounds. A group of seven 35-year-old cedars at Cowichan Lake, Vancouver Island, were completely rotted by *Poria subacida*. This decay extended up the trees approximately 10 feet.

Another observation of some interest was on the condition of young, oppressed trees, which are of frequent occurrence in most forest stands containing cedar. Although no systematic sampling was carried out, it was found that a large number of the trees that were inspected were badly decayed. *Poria asiatica*, *P. Weirii*, *P. subacida*, *P. albipellucida*, *Fomes Pini*, and a *Merulius*-like fungus all have been cultured from rot in such trees.

### Decay of Logs and Slash

All of the fungi causing decay in living cedars are apparently capable of continuing decay in felled trees and logs, while many of these are also capable of decaying slash or primarily live on slash. The greatest damage is probably caused by *Poria Weirii*, *P. albipellucida*, and *P. subacida*. *Fomes pinicola*, *F. annosus*, and *F. applanatus* have been collected fruiting on cedar logs, but the exceedingly limited reports of these three species of *Fomes* on western red cedar would indicate that their occurrence is only of an incidental nature.

According to Murrill (21), *Poria Weirii* "continues the destruction of the heartwood and later of the sapwood after the tree has fallen and may extend along the entire tree, even attacking the bark." Little has been observed concerning the activity of *P. albipellucida* in felled trees and logs of cedar. It has been found, however, that in hewn railroad ties, culled for laminate heart rot caused by this fungus, the organism has continued growth through the tie, and in one case fruited on the lower side. *P. subacida* is frequently found as an active decay organism of all parts of small logs and immature cedar. It is on such logs that the conks of the fungus most frequently occur.

Two fungi, *Polyporus cuneatus* (Murr.) Overh. and *Hymenochaete tabacina* (Sow.) Lév., have been found widely distributed, causing decay of cedar sapwood and slash. Other species, such as *Polyporus abietinus* (Dicks.) Fr., *P. versicolor* (L.) Fr., *P. semipileatus* Peck, *P. hirsutus* (Wulf.) Fr., and *Poria isabellina* (Fr.) Overh., have been found restricted in distribution or of irregular occurrence. Still others, particularly species of Agaricaceae and Thelephoraceae, appear to be only of incidental or rare occurrence on cedar sapwood and slash.

*Polyporus cuneatus* (4) is the most important single organism responsible for decay of cedar sapwood and slash (7). Throughout all regions investigated this fungus was found fruiting on logs and branches, most frequently inhabiting densely shaded, moist logs, and growing through the bark seams to form the typical imbricate or resupinate conks. It is also found attacking decorticated areas of dead or living trees. *P. cuneatus* causes a white spongy rot of the sapwood of cedar. *Hymenochaete tabacina* is very general in distribution but of much less importance than *Polyporus cuneatus*. This fungus was found chiefly on dead branches and occasionally on larger material. Except as a decay of slash this fungus is of no economic importance.

*P. abietinus*, *P. semipileatus*, and *P. hirsutus* were found only in the Interior, while *Poria isabellina* and *Polyporus versicolor* were found only on the Coast, but probably occur in the Interior also. *P. abietinus* was frequently found growing on logs and branches from the bark seams in a similar habitat and manner to *P. cuneatus*. *P. semipileatus* occurred less frequently than *P. abietinus* but may cause extensive decay of sapwood in localized areas. *P. hirsutus* was found only on cedar logs still retaining the bark and in much dryer situations than the other sap rot organisms. All these fungi cause white to yellow, spongy to fibrous sap rots. *Poria isabellina* was frequent on decorticated cedar logs, causing a yellowish pitted rot of the sapwood. This fungus was commonly associated with decay directly beneath the charcoal on the surface of burned logs. *Polyporus versicolor* has been found only to a very limited extent during the course of this study.

Little economic importance is attached to the decays of cedar sapwood and slash. Some loss through decay of the sapwood of cedar poles incorrectly stored, however, might warrant further investigation of the causative fungi at some future date.



### Cultural Characteristics of the Fungi

Most of the causal organisms of the decays investigated in this study were identified by making isolations of the fungi from incipient decay and comparing the cultures with those of known fungi. It was found that the fungus in a piece of inoculum did not grow on to the media as successfully on malt agar as it did on potato-dextrose agar. After the fungus was established on potato-dextrose agar, however, it was found that when a transfer was made to malt agar the fungus grew as well as on the former medium.

The isolations obtained from the decays were grown on tannic and gallic acid media for grouping and partial identification. Table III gives the reactions and growth of the fungi of importance in this study on acid media

TABLE III

REACTION AND GROWTH OF THE MAJOR WOOD-ROTTING FUNGI OF WESTERN RED CEDAR ON GALLIC AND TANNIC ACID MEDIA AT THE END OF SEVEN DAYS

Fungus	Isola- tions	On gallic acid medium		On tannic acid medium	
		Reaction*	Growth, mm.	Reaction*	Growth, mm.
White-rot fungi:					
<i>Poria albipellucida</i>	45	++	40-90	+++	0-20
<i>Poria cinerescens</i>	2	++	50-60	+++	0-tr.**
<i>Poria subacida</i>	5	++++	0-14	+++	20-40
<i>Poria Weirii</i>	2	+++	25-35	+++	15-20
<i>Fomes Pini</i>	13	++++	Tr.-10	+++	Tr.-15
<i>Fomes annosus</i>	6	++++	0	++++	10-20
<i>Omphalia campanella</i>	7	++++	0-tr.	+++	0-tr.
Brown-rot fungi:					
<i>Poria asiatica</i>	45	-to+†	10-20	-to+††	0-tr.
<i>Polyporus balsameus</i>	5	-to+†	10-20	-to+††	0-tr.
<i>Merulius "A"</i>	8	—	20-30	—	0-tr.
<i>Merulius "B"</i>	14	—	10-20	—	0-tr.

\* — = Negative, no brown discoloration of agar.

+ = Diffusion zone light to dark brown, formed under inoculum at centre of mat and visible only from underside of dish.

++ = Diffusion zone light to dark brown, formed under most of mat but not extending to the margin.

+++ = Diffusion zone light to dark brown, extending a short distance beyond the margin of the mat.

++++ = Diffusion zone dark brown, opaque, extending considerably beyond the margin of the mat.

\*\* Trace of growth only.

† Reaction usually negative.

†† Reaction usually a slight brown diffusion zone under the inoculum.

at the end of seven days, following the procedure outlined by Davidson, Campbell, and Blaisdell (11). Using this method, it was not possible to find consistent differential characteristics between *Poria albipellucida* and *P.*

*cinerescens* (Bres.) Sacc., and between *Poria asiatica* and *Polyporus balsameus*. It was found, also, that the cultures ascribed to *Merulius* sp. could be divided into two types, "A" and "B," on the basis of their reactions.

Although the description of the conks of *Poria albipellucida* (3) and *P. cinerescens* (23) would readily separate these organisms, it is on rare occasions only that the conks are found, so most identifications must be made from cultures. No way has been found to date to separate the cultures of these two organisms. On tannic and gallic acid media their reactions are similar and very distinctive. On malt agar their growth is rapid, and certain habits of growth would readily distinguish these two organisms from other fungi. In one week the agar in the Petri dish is covered by a thin, white, appressed mat, showing inverted 'V' formations where the mycelium is thickened (Pl. V, Fig. 5). No fruit body of *P. cinerescens* has been found on cedar during this investigation, so all the white laminate decays, from which these distinctive cultures have been isolated, are ascribed to *P. albipellucida*. It is interesting to note, however, that the Department of Agriculture, Ottawa, Mycological Herbarium contains no sporophores of *P. albipellucida* from cedar in British Columbia, but has specimens of *P. cinerescens* from cedar logs from which the remarkably similar cultures to *P. albipellucida* were obtained.

*Polyporus balsameus* (16) and *Poria asiatica* (22) are readily separated by a comparison of the sporophores and by the colour of the cultures, but the great similarity in the appearance of the decays led to the discovery of some striking similarities in culture. In very young cultures these two species cannot usually be separated at present and even after some time the similarity in growth habits between the cultures of the two organisms is close. The only point of differentiation found to date between the two organisms in culture is that the chlamydospores of *P. asiatica* remain hyaline, while those of *Polyporus balsameus* darken, giving rise to the previously mentioned difference in the colour of cultures. Cultures of the two organisms from the Interior have fruited.

Whether two organisms are involved in the *Merulius* complex or whether the one species shows considerable variation is not certain. On gallic and tannic acid media it is possible to separate the cultures into two forms: *Merulius* "A," which grows rapidly across the gallic acid medium with long, feathery, mycelial processes (Pl. V, Figs. 6 and 7), and *Merulius* "B," which grows in a soft appressed mat, 'feathering' only slightly at the edges (Pl. V, Fig. 8). Both forms exude numerous droplets of clear fluid that turns yellow with age.

*Omphalia campanella* has fruited on three occasions in culture, once from the original inoculum on potato-dextrose agar and twice on a medium suggested by Badcock (1). This fungus is easily distinguished in culture as it commonly produces 'aerial rhizomorphs.'

# Check List of Fungi Occurring on Cedar

During the course of this investigation, it was found advisable to collect all fungi fruiting on any portion of red cedar. Not only did such a procedure build up a check list of fungi occurring on cedar, but for some of the decays it uncovered the fruiting body of the causal organism, occasionally small and insignificant in character, and thus made the identification of the decay and fungus possible. Such was the case with *Poria albipellucida*. The following check list, Table IV, represents all the Basidiomycetes found on living and dead western red cedar in British Columbia from 1943 to 1945.

TABLE IV  
CHECK LIST OF FUNGI COLLECTED ON WESTERN RED CEDAR IN  
BRITISH COLUMBIA FROM 1943 TO 1945

Species	Locality of collections
<i>Armillaria mellea</i> (Fr.) Quél.	12, 13, 18*
<i>Auricularia auricularis</i> (Gray) Martin	13
<i>Collybia</i> sp.	12
<i>Coniophora betulae</i> (Schum.) Karst.	13
<i>Coniophora puteana</i> (Schum. ex Fr.) Karst.	16
<i>Coniophora suffocata</i> (Peck) Massee	16
<i>Corticium</i> sp.	1, 3, 7, 11
<i>Corticium bicolor</i> Peck	3
<i>Corticium cebennense</i> Bourd.	3
<i>Corticium coronilla</i> Höhn.	3
<i>Corticium livido-caeruleum</i> Karst.	1, 3
<i>Corticium racemosum</i> Burt.	12
<i>Corticium radiosum</i> Fr.	12
<i>Corticium sulphureum</i> (Pers. ex Fr.) Fr.	1
<i>Crepidotus herbarum</i> Peck	17
<i>Flammula decorata</i> Murr.	17
<i>Flammula liquiritiae</i> (Weinm.) Quél.	11
<i>Fomes applanatus</i> (Pers.) Wallr.	3
<i>Fomes annosus</i> (Fr.) Cke.	1, 3, 7, 9
<i>Fomes nigrolimitatus</i> (Romell) Egel.	7, 11
<i>Fomes Pini</i> (Thore) Lloyd and var. <i>abietis</i> Karst.	5, 9, 11, 12, 13, 15
<i>Fomes pinicola</i> (Swartz) Cke.	1, 2, 10
<i>Gloeocystidium ochroleucum</i> Bres.	1
<i>Helvella</i> sp.	3
<i>Hypholoma fasciculare</i> (Fr.) Quél.	3
<i>Hypholoma capnoides</i> (Fr.) Quél.	1
<i>Hymenochaete fuliginosa</i> (Pers.) Bres.	13
<i>Hymenochaete tabacina</i> (Sow.) Lév.	1, 3, 5, 12, 13, 18
<i>Lenzites saepiaria</i> (Wulf.) Fr.	16

\* The numbers refer to localities on the map, Text-fig. 1.

TABLE IV

CHECK LIST OF FUNGI COLLECTED ON WESTERN RED CEDAR IN  
BRITISH COLUMBIA FROM 1943 TO 1945—*Concluded*

Species	Locality of collections
<i>Marasmius scorodonius</i> Fr.	
<i>Merulius fugax</i> Fr.	
<i>Mycena griseiconica</i> Kauffm.	
<i>Odontia</i> sp.	1, 3, 11
<i>Odontia alutacea</i> (Fr.) B. & G.	1
<i>Odontia alutacea</i> subsp. <i>floccosa</i> B. & G.?	16
<i>Odontia aspera</i> (Fr.) Bourd.	9
<i>Odontia lactea</i> Karst.	12, 13
<i>Omphalia campanella</i> (Fr.) Quél.	4, 7, 8, 12, 15, 18
<i>Peniophora</i> sp.	1
<i>Peniophora crassa</i> Burt.	1
<i>Peniophora flavo-ferruginea</i> Karst.	1
<i>Peniophora sanguinea</i> (Fr.) Bres.	1
<i>Peniophora velutina</i> (DC) Cke.?	1
<i>Phlebia mellea</i> Overh.	13
<i>Polyporus abietinus</i> (Dicks.) Fr.	13, 15, 18
<i>Polyporus balsameus</i> Peck	12, 15, 17
<i>Polyporus caesius</i> (Schrad.) Fr.	19
<i>Polyporus cuneatus</i> (Murr.) Overh.	5, 7, 11, 12, 13, 14, 15, 17, 18
<i>Polyporus dichrous</i> Fr.	13, 16, 18
<i>Polyporus elegans</i> (Bull.) Fr.	3, 12
<i>Polyporus hirsutus</i> (Wulf.) Fr.	16, 18
<i>Polyporus immilis</i> Peck	10
<i>Polyporus perennis</i> (L.) Fr.	5
<i>Polyporus Schweinitzii</i> Fr.	5
<i>Polyporus semipileatus</i> Peck	13, 15, 18
<i>Polyporus undosus</i> Peck	1
<i>Polyporus versicolor</i> (L.) Fr.	5
<i>Poria</i> sp.	, 13
<i>Poria albipellucida</i> Baxter	, 2, 3, 4, 5, 6, 7, 8, 11, 13
<i>Poria asiatica</i> (Pilát) Overh.	, 4, 5, 6, 7, 8, 12, 14, 15, 18, 19
<i>Poria candidissima</i> (Schw.) Cke.	
<i>Poria isabellina</i> (Fr.) Overh.	, 5, 6
<i>Poria lenis</i> Karst.	
<i>Poria nigrescens</i> Bres.	11
<i>Poria sericeo-mollis</i> (Romell) Baxter	1
<i>Poria sinuosa</i> (Fr.) Sacc.	11
<i>Poria subacida</i> (Peck) Sacc.	1, 2, 3, 5, 7, 11, 12, 13, 18
<i>Poria Weirii</i> Murr.	1, 3, 4, 8, 9, 10, 12, 13, 15, 17, 18
<i>Psathyrella</i> sp.	11
<i>Schizophyllum commune</i> Fr.	1
<i>Stereum Chaillatii</i> Pers.	3
<i>Stereum rugosiusculum</i> B. & C.	3
<i>Stereum sanguinolentum</i> Alb. & Schw.	18
<i>Tomentella</i> sp.	1
<i>Trametes carbonaria</i> (B. & C.) Overh.	1, 16, 19
<i>Trametes mollis</i> (Sommerf.) Fr.	13
<i>Trametes sepium</i> Berk.?	19

## Discussion

The problem of determining the causal fungi of the decays of western red cedar was found to be very difficult. This difficulty made it necessary to stress the collection of fungi fruiting on cedar and the inspection of trees from which large specimens of decay were procurable. The collection and study of discoloured material and the restriction of the investigation to areas where data on total age and decay correlations could be collected were not attempted. All investigations were directed solely to decays and causal fungi.

This investigation, because of the foregoing restrictions in the material studied, uncovered little information of value in the management plans for western red cedar. A study of the figures gathered for the loss from decay in cedar would suggest that decay was never a factor of importance affecting the silviculture and management of the species. This is not believed to be true, however. The figures represent the condition of mature cedar trees selected by the logger or pole-cutter as probably containing a merchantable volume of sound wood or a pole. When considering the possibility of managing young cedar stands, the previous remarks concerning the attack of immature trees must be weighed. In these discussions it is seen that young cedars are apparently quite susceptible to attack by several important heart-rotting organisms. Until a careful study is made of the conditions existing in these young trees, no conclusions can be drawn concerning the significance of decay in its effect on the silviculture and management of red cedar. Observational evidence would indicate that decay in young cedars is of importance and should be studied carefully in relation to the management of the species.

*Poria albipellucida* and *P. Weirii* occur on the Coast and in the Interior but as heart-rotting organisms the former is only important on the Coast and the latter in the Interior. The reason for this is not clear.

The obscure picture of the decay complex on cedar has not been entirely cleared up by this investigation. It is felt, however, that the principal fungi responsible for the decay in mature trees and an indication of their relative importance, have been determined. Much work remains to be done to bring the knowledge of cedar decays and defects to a plane equal to that of other tree species of major economic importance in British Columbia.

## Acknowledgments

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## ON STAINING FOR VITAMIN C IN TISSUES<sup>1</sup>

BY JULES TUBA<sup>2</sup>, GEORGE HUNTER<sup>3</sup>, AND JOHN A. OSBORNE<sup>4</sup>

### Abstract

Guinea-pig adrenals were stained for ascorbic acid. In the normal adrenal, staining was found in both cortex and medulla though with greatest density in the zona fasciculata. The stained scorbutic adrenal showed only a few granules.

The mosses *Mnium cuspidatum* and *Mnium affine* contain ascorbic acid in appreciable amounts. Following staining, fine black granules could be seen in both species. The stain intensity of the granules roughly corresponded to their relative ascorbic acid content.

Having satisfactorily established the validity of the staining solution by the above experiments the development of ascorbic acid in the rose hips of *R. acicularis* was followed throughout the summer. There was found to be some association between the ascorbic acid and chloroplasts. With increasing maturity there developed a gradual accumulation of fine granules in the cells of the tissue as well. These granules showed no apparent relationship to any formed elements in the cells. Staining densities roughly parallel ascorbic acid content.

### Introduction

Since Szent-Györgyi (8) observed that the suprarenal cortex reduces silver nitrate, many workers have studied the effects on various tissues that are known to contain vitamin C. Silver nitrate is very easily reduced in ammoniacal solution, much less readily in neutral solution, and in acid solution with comparative difficulty. It does not undergo reduction in solutions of organic acids such as malic, tartaric, citric, and malonic, even when boiled (6) unless there be present in addition an oxidizing agent such as potassium permanganate. Leblond (5) subsequently showed that strongly reducing substances such as adrenalin, polyphenols, various sugars, and cysteine have no effect on acid silver nitrate solution. In developing a technique of staining for vitamin C in tissues of the rat, guinea-pig, and mouse, Bourne (1) found that the only substance other than vitamin C capable of giving a black precipitate with acid silver nitrate in the dark is hydrogen sulphide.

The most important evidence for the specificity of acid silver nitrate staining is obtained from scorbutic and non-scorbutic tissues. Investigators, including Leblond (5), Bourne (1), Gough and Zilva (4), Westergaard (10), Giroud and Leblond (3), Giroud (2), have stained a great variety of tissue, including that of the adrenal, testis, kidney, pituitary, and ovary. It was found that the depth of staining decreased progressively with the development of ascorbic acid deficiency.

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## Experimental

### Reagents

The staining solution of Smyth, Bingley, and Hill (7) was used. This is prepared by adding 5 ml. of 5% acetic acid solution to 100 ml. of 5% silver nitrate solution. A 5% solution of sodium hyposulphite was used to remove the unreduced silver nitrate. Analytically pure silver nitrate and sodium hyposulphite were used in the preparation of the above reagents. The staining solution and the hyposulphite solution were prepared daily and, when not in use, they were kept in a refrigerator.

### Materials

Our main concern was the determination of the histological distribution of vitamin C in rose hips and these constitute the main materials used. In the development of technique and to obtain evidence for the validity of the staining, we had recourse to the use of other plant materials, including several species of mosses, and, in addition, adrenal glands from normal and scorbutic guinea-pigs.

### Methods

Preliminary experiments with iris leaves and green rose hips showed that plant material is more resistant than animal tissue to penetration by acid silver nitrate solution. For example, intact guinea-pig adrenal glands can be immersed in the staining solution, the excess silver nitrate washed out with sodium hyposulphite solution, and then the tissue, after dehydration, can be embedded and sectioned by routine histological methods. The satisfactory result obtained in this way is shown in Fig. 1. However, the acid silver nitrate will not penetrate similar masses of plant tissue, and it was necessary to modify the staining technique to overcome this difficulty.

The moss, *Mnium*, has leaves that are only one cell thick. Hence, these may be readily stained and examined without sectioning. Figs. 3 and 4 show the presence of fine black granules in concentrations that roughly parallel the vitamin C content.

However, sectioning was necessary to determine the distributions of ascorbic acid in rose hips. Sectioning the fresh material and subsequent staining of the individual sections was accompanied by much loss of the vitamin, probably due to leaching, and by the formation of artifacts stained with the silver nitrate.

After considerable trial and error the following method was adopted. The whole fresh rose hip is embedded in paraffin and mounted on a sliding microtome. The block is sectioned until the top third of the hip is cut away. The block is then immediately immersed in the staining solution where it is left, in the dark, at room temperature for 30 min. The block is now washed with sodium hyposulphite solution. Excess moisture is then removed and sectioning is continued. Sufficient penetration of the tissue by the staining solution is obtained in this way to yield several satisfactorily stained sections. Sections are mounted in Farrant's solution for microscopical examination.



The thickness of the sections varied from 15 to 45  $\mu$ , directly with the increasing maturity of the rose hips. As the hips increase in size, the tissue cells increase proportionately in diameter. The thickness of the sections is kept greater than the diameter of the largest cells, in order that there should always be present intact cells. Otherwise it is impossible to get continuous sections of the soft, ripe hips in the unfrozen condition. Freezing would not have solved this problem, because there would still have remained the possibility of some of the black, stained material escaping from the sectioned cells.

The staining method here described, with such variable section thickness, can make no claim to quantitative precision in estimating the ascorbic acid content of the tissue. However, this variation is to a large extent compensated for by the uniform and short depth of focus of the microscope, so that the plates do show a fair parallelism between the intensity of staining and the titration values obtained with 2,6-dichlorophenolindophenol for vitamin C in control samples.

## Discussion of Results

### *Adrenal Glands*

In confirmation of the silver staining for vitamin C in animal tissues, Figs. 1 and 2, illustrating conditions in the cortex of the adrenals of normal and scorbutic guinea-pigs, may be referred to. The normal adrenal, with a dye titration value of 109 mgm. ascorbic acid per 100 gm., shows the occurrence in considerable frequency of fine black granules. In the scorbutic animal, with less than 24 mgm. ascorbic acid per 100 gm. adrenal, the stained gland shows only a few granules.

The area photographed in Fig. 1 is in the zona fasciculata, immediately below the zona glomerulosa; in Fig. 2, the area is the same with the zona glomerulosa showing on a boundary distorted in mounting.

In the normal adrenal, staining is found in both cortex and medulla, though most abundantly in the zona fasciculata of the cortex.

The concentration of granules into streaks in the upper region of Fig. 1 is not regarded as typical for the zona fasciculata.

That scurvy was well advanced in our scorbutic animal was proved by bone sections that showed extensive haemorrhage in the marrow and characteristic loss of matrix and degeneration of bone cells.

FIG. 1. Section of normal guinea-pig adrenal gland, 109 mgm. vitamin C/100 gm., through zona fasciculata.  $\times 320$ .

FIG. 2. Section of scorbutic guinea-pig adrenal gland, less than 24 mgm. vitamin C/100 gm., through zona glomerulosa and fasciculata.  $\times 320$ .

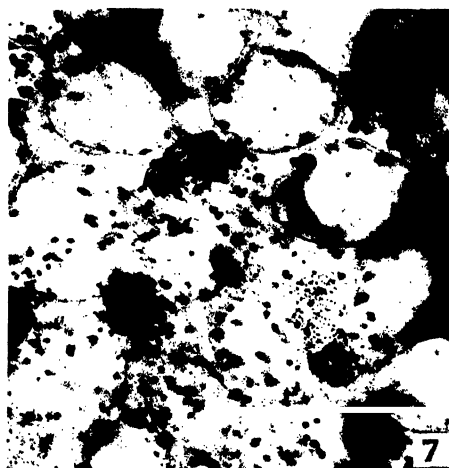
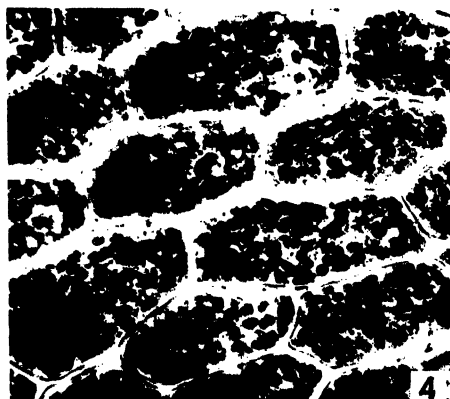
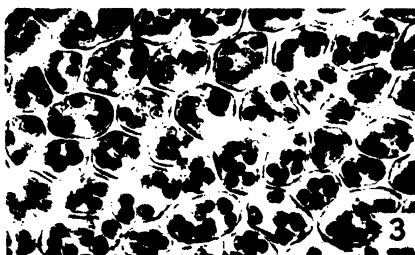
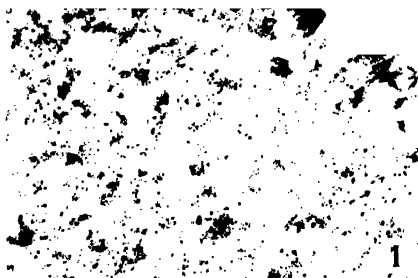
FIG. 3. Section of *Mnium cuspidatum* leaf, 33 mgm. vitamin C/100 gm.  $\times 320$ .

FIG. 4. Section of *Mnium affine* leaf, 130 mgm. vitamin C/100 gm.  $\times 320$ .

FIG. 5. Section of rose hip, 140 mgm. vitamin C/100 gm.  $\times 320$ .

FIG. 6. Section of rose hip, 300 mgm. vitamin C/100 gm.  $\times 80$ .

FIG. 7. Section of rose hip, 300 mgm. vitamin C/100 gm.  $\times 320$ .





### Mosses

The mosses, *Mnium cuspidatum* (L.) Leyss and *Mnium affine* Bland, illustrated in Figs. 3 and 4, are tissues one cell thick but with a marked difference in size of the cells in the two species. Fine black specks can be seen in both species, but more abundantly in *Mnium affine*, which by titration proved to have nearly four times the concentration of ascorbic acid shown by *Mnium cuspidatum*.

The greater size of the cells of *Mnium affine* of course makes strict comparison of the staining in the two species impossible, but the very small depth in the range of focus eliminates to a large extent the differences in the frequency of the black specks that may be due to the different thicknesses of the leaves.

It is evident in both Figs. 3 and 4 that the chloroplasts are not stained. The black specks occur quite distinct from the chloroplasts. Nor is there staining in the intercellular spaces. It would thus seem correct to conclude that vitamin C exists in aggregates in the cytoplasm of these mosses. A search was made in the fresh leaves, with and without cross nicols, for the possible existence of crystals of ascorbic acid, but such were not found.

It may also be noted that no bodies, corresponding to the black specks found after staining with silver, could be found in the fresh leaves, or in those stained with haematoxylin and eosin.

Various other mosses that contained no vitamin by titration were stained. The leaves of most of these were more difficult to examine without sectioning, but in no case where the titration was zero were any black granules discernible.

Table I records our observations with mosses.

It may be noted that at an earlier date we assayed a number of mosses for vitamin C with uniformly negative results (9). This subject is of some interest in relation to vitamin C sources in the Arctic.

### Rose Hips

Our observations were confined to *Rosa acicularis* Lindl. The hips were picked throughout the season of development, from the small green preflower stage to the red ripe stage. Table II gives the titration values for material used in Figs. 5 to 12.

Fig. 5 shows conditions at an early stage of development of the rose hip, with small cells, poorly developed cell walls, black stained chloroplasts, and a relatively low ascorbic acid content of 142 mgm./100 gm. of tissue. The round or oval black bodies are certainly chloroplasts that show up in similar sections stained with haematoxylin and eosin. There are few, if any, fine black specks such as were found in the adrenal gland, or in the *Mnium* mosses. It would thus appear that at this stage the vitamin C is definitely associated with the chloroplasts.

At a later stage, Figs. 6 and 7, when the vitamin C has more than doubled its concentration, and the cells have increased in size and definition, there appear besides the black stained chloroplasts, finer black bodies that are not chloroplasts, and that resemble the black specks in the normal adrenal and

*Mnium* mosses. In both sections there are cells showing marked aggregation of black stained material that appears to be a mixture of stained chloroplasts and non-chloroplastic black granules.

TABLE I  
THE VITAMIN C CONTENT OF VARIOUS ALBERTA MOSSES

Date	Name of moss	Vitamin C by dye titration, mgm./100 gm.	Result where stained
June 27, 1945	<i>Mnium affine</i> Bland	266	+
June 29	<i>Mnium affine</i> Bland	254	+
June 30	<i>Mnium affine</i> Bland	213	+
June 30	<i>Mnium affine</i> Bland	179	+
Aug. 10	<i>Mnium affine</i> Bland	133	Fig. 4
July 11	<i>Mnium cuspidatum</i>	34	+
Aug. 7	<i>Mnium cuspidatum</i>	26	+
Aug. 7	<i>Mnium cuspidatum</i>	49	+
Aug. 7	<i>Mnium cuspidatum</i>	33	+
Aug. 7	<i>Mnium cuspidatum</i>	13	+
Aug. 7	<i>Mnium cuspidatum</i>	35	+
Aug. 7	<i>Mnium cuspidatum</i>	33	Fig. 3
Aug. 10	<i>Mnium cuspidatum</i>	50	+
June 30	<i>Polytrichum juniperinum</i>	25	?
June 30	<i>Funaria hygrometrica</i>	28	+
June 30	<i>Aulacomnium palustre</i>	0	0
June 30	<i>Sphagnum fuscum</i>	0	0
June 30	<i>Sphagnum capillaceum tenellum</i>	0	0
June 30	<i>Sphagnum magellanicum</i>	0	0
June 30	<i>Sphagnum tenerum</i>	0	0

TABLE II  
THE VITAMIN C CONTENT IN RELATION TO STAGE OF DEVELOPMENT

Season	Vitamin C by dye titration, mgm./100 gm.	Stage of development	Figure No.
Spring	142	Preflower, green	5
	305		6, 7
	540		8, 9
Mid summer	848	Postflower, green	10
	972		
	1007		
	1161		
Late summer	1424	Ripe hip	11, 12

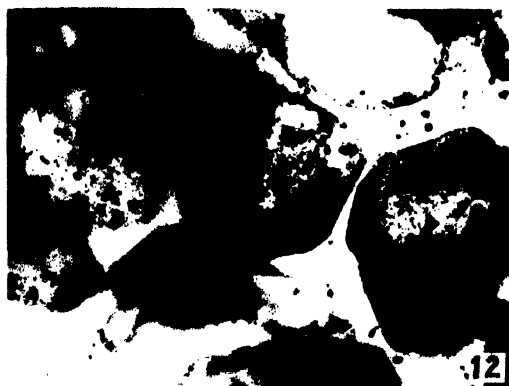
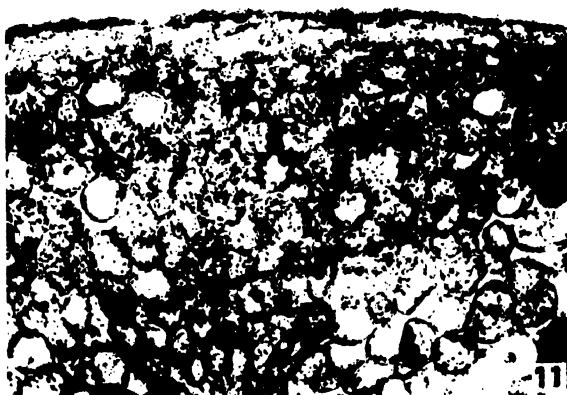
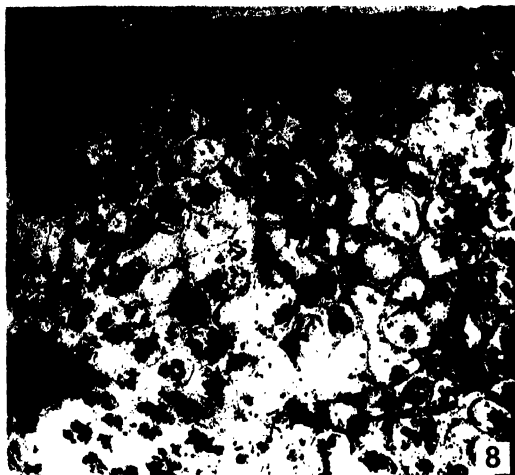
FIG. 8. Section of rose hip, 540 mgm. vitamin C/100 gm.  $\times 80$ .

FIG. 9. Section of rose hip, 540 mgm. vitamin C/100 gm.  $\times 320$ .

FIG. 10. Section of rose hip, 972 mgm. vitamin C/100 gm.  $\times 320$ .

FIG. 11. Section of rose hip, 1424 mgm. vitamin C/100 gm.  $\times 80$ .

FIG. 12. Section of rose hip, 1424 mgm. vitamin C/100 gm.  $\times 320$ .





When the hips are still in the preflower stage but the vitamin C concentration has again almost doubled (Figs. 8 and 9), a comparison of Figs. 6 and 8 shows a more generalized staining corresponding to the increased vitamin C content. The chloroplasts are less regular and the proportion of fine granules has increased.

In Fig. 10, when the flower has been shed, and the hip is green and about three-quarters its mature size and the vitamin C has increased in concentration to 972 mgm./100 gm., the preponderance of fine granules and the formation of dense stained aggregates in the cells are increased in prominence.

Figs. 11 and 12 represent conditions in the ripe stage when the vitamin reaches 1424 mgm./100 gm. The low power picture (Fig. 11) may be compared with the earlier stages (Figs. 8 and 6) to see the increased generalized staining with increased vitamin C concentration. Under the high power the intact cells are seen to be large and only partially in focus. There appear to be some regularly shaped chloroplasts still left but the majority of the chloroplasts appear in process of degeneration, assuming reticular shapes, showing extrusions and extensions that may be aggregates of fine granules. Fine black discrete granules are abundant, as in the central cell of Fig. 12.

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The authors wish to express their thanks to Dr. R. F. Shaner and Mr. A. G. Fairall, Department of Anatomy, for their constructive criticism of histological technique, and to Dr. E. H. Moss, Department of Botany, for collecting and identifying the mosses, and for information regarding plant tissues.

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# CULTURAL AND TAXONOMIC STUDIES ON CERTAIN FUSARIA

## I. MUTATION IN CULTURE<sup>1</sup>

BY J. J. MILLER<sup>2</sup>

### Abstract

This work represents a continuation of previous studies by the writer on the *Fusarium* of muskmelon wilt, with a view to determining the extent to which the cultural phenomena noted in this organism are characteristic of other *Fusaria*. The section *Elegans* is represented by 13 organisms, and the sections *Liseola*, *Discolor*, and *Gibbosum* by one each. Abnormal areas appearing in cultures of these organisms were investigated from the genetic viewpoint, and the results showed the larger of these to be of mutant origin. Evidence was obtained that the 'sclerotia' that appeared in cultures of some of the organisms may likewise be of mutant origin, but this was not deemed conclusive. A comparison of the properties attributed in the literature to 'sporodochia' appearing in *Fusarium* cultures with those of the patch mutants studied here indicates that the terms are synonymous in this case, and hence the common practice in *Fusarium* taxonomy of measuring 'macroconidia from sporodochia' is questioned. Since the wild types of the *Fusaria* studied were readily displaced in culture by mutants, it is evident that some method of maintaining the original isolates in the pure state is fundamental to a consistent taxonomy of the genus. In this connection the practice of maintaining *Fusaria* in tubes of sterilized soil is recommended, since experiments showed that this type of cultural treatment does not favour the loss of the wild type. A discussion is given concerning the cause of variation and the extent to which it occurs in nature.

### Introduction

In previous work on the *Fusarium* of muskmelon wilt already described (23, 24) it was pointed out that initially all the isolates of the organism obtained from plants diseased in naturally infested soil were alike in culture. All produced abundant aerial mycelium on which small non-septate conidia were borne rather sparsely. This cultural form was termed the 'wild type.' When grown in culture the wild type was found to be readily displaced by mutants, and this process could be favoured by transferring the cultures successively to fresh slants. Dense structures appeared frequently in cultures of the wild type. Since monosporous cultures obtained from such areas consistently included a high proportion that were mutant, they were termed 'patch mutants.'

The present studies involve a continuation of this work with a view to determining whether the cultural behaviour observed is also shown by other members of the genus.

### Materials and Methods

The 16 *Fusaria* employed during the present studies were obtained from various sources, as outlined in Table I. Material was received either as freshly isolated cultures or as diseased host material from which isolations

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TABLE I

SUMMARY OF THE CLASSIFICATION AND ORIGINS OF THE *Fusarium* ISOLATES STUDIED

Organism	Section of genus	Host	Place of origin	Date of isolation	Contributor
Muskmelon wilt <i>Fusarium</i> *	Elegans	<i>Cucumis Melo</i> L.	Ontario	1942, 1943 1944, 1945	R. G. Atkinson
Pea root rot <i>Fusarium</i> †	Elegans	<i>Pisum sativum</i> L.	Ontario	1945	
Soybean blight <i>Fusarium</i> *	Elegans	<i>Glycine max</i> Merr.	Ontario	1944	
Tomato wilt <i>Fusarium</i> *	Elegans	<i>Lycopersicon esculentum</i> Mill.	Ontario	1944	
Aster wilt <i>Fusarium</i>	Elegans	<i>Callistephus</i> sp.	Ontario	1944	
Navy bean root rot <i>Fusarium</i> *†	Elegans	<i>Phaseolus vulgaris</i> L.	Ontario	1944	D. L. Bailey
Gladiolus vascular <i>Fusarium</i>	Elegans	<i>Gladiolus</i> sp.	Ontario	1945	
Cabbage wilt <i>Fusarium</i>	Elegans	<i>Brassica oleracea</i> L.	Ontario	1945	
Flax wilt <i>Fusarium</i>	Elegans	<i>Linum usitatissimum</i> L.	Ontario	1944, 1945	
Cotton wilt <i>Fusarium</i>	Elegans	<i>Gossypium</i> sp.	Texas	1944	
Cowpea wilt <i>Fusarium</i>	Elegans	<i>Vigna sinensis</i> Endl.	Texas	1944	G. E. Altstatt
Soil <i>Fusarium</i> A	Elegans		Ontario	1945	
Soil <i>Fusarium</i> B	Elegans		Ontario	1945	
<i>Fusarium moniliforme</i> Sheldon*	Liseola	<i>Zea mays</i> L.	Ontario	1945	
<i>Gibberella Saubinetii</i> (Mont.) Sacc.	Discolor	<i>Triticum aestivum</i> L.	Ontario	1945	
<i>Fusarium Scirpi</i> Lamb. & Fautr.††	Gibbosum	<i>Glycine max</i> Merr.	Ontario	1945	

\* Pathogenicity confirmed by infection experiments.

† On the basis of host relationship and symptomatology these *Fusaria* would appear to be closely similar to two pathogens described by Snyder (33) as belonging to the section *Martiella*. However, they were found here to be morphologically so similar to the *Elegans* *Fusaria* under study that there seems no question that they belong in this section. The aster and gladiolus *Fusaria*, for instance, were found to be morphologically more similar to the navy bean *Fusarium* than to the muskmelon or cabbage *Fusaria*.

†† It is not believed that this organism, found fruiting on the root of a diseased soybean plant, was growing as a parasite since the unhealthy condition of the plant appeared due to other causes.

were made by the writer. Two *Fusaria* of the *Elegans* type, presumably saprophytes, were isolated from soil by the plate method and for convenience are referred to as Soil *Fusarium* A and Soil *Fusarium* B. No specific names have been attached to any of the *Elegans* *Fusaria* studied. Although it may seem advisable to designate the organisms by some commonly employed system of nomenclature, in view of the conclusions reached in this series concerning the taxonomic situation in the genus, neither the monograph of Wollenweber and Reinking (43) nor the recent revision of the *Elegans* *Fusaria* proposed by Snyder and Hansen (34) can be regarded as satisfactory. As will be apparent from a publication to follow, the 'normal' conidium technique upon which the Wollenweber and Reinking monograph is mainly based precludes the identification of the *Elegans* *Fusaria* studied here with any of those described in "Die Fusarien." The Snyder and Hansen revision disregards morphological criteria and places primary emphasis on the host relationship. This is not in agreement with the conclusion reached during

the present studies, that if proper cultural methods are employed the morphology of these organisms will be found sufficiently constant to warrant an attempt at morphological classification. Hence it is felt that the practice adopted in Table I of listing the host and the type of disease caused by a given organism cannot be improved upon at the present time. In the case of the last three *Fusaria* listed in the table, the nomenclature of "Die Fusarien" has been followed although, except in the case of *F. moniliforme*, it was not found possible to reach a satisfactory determination on morphological grounds. *Gibberella Saubinetii* was identified as such mainly on the basis of host relationship and symptomatology. The organism designated as *F. Scirpi*, owing to the elongate terminal cells of the conidia it produced on the soybean plant, apparently belongs in Wollenweber's section *Gibbosum*, and it was found to fit more closely the description of the above-mentioned organism than that of any other given in the section. It should be pointed out that the pathogenicity of all the organisms studied has not been confirmed by infection experiments and the possibility exists that some of these were saprophytes and were not attacking the host in question. This would not be a serious drawback as far as the present studies are concerned since these organisms were employed mainly to determine whether other *Fusaria* display a cultural behaviour similar to that of the muskmelon pathogen.

Stock cultures of the organisms were prepared in the following manner: shortly after isolation each organism was purified by the single spore technique and tubes of moist sandy loam soil (sterilized) were inoculated from young single spore cultures of each. The contents of these tubes slowly became dry and soil grains from them were employed during subsequent experiments as a source of inoculum, a practice that was justified by the results of an experiment on cultural degradation to be described.

The technique followed for isolating single spores has been outlined previously (23). The nutrient medium most frequently employed was potato dextrose agar prepared according to the formula of Riker and Riker (28). Soil agar (or, more correctly, soil infusion agar) was prepared from sandy loam soil according to the method previously described (23).

The nomenclature employed here for the mutants is that of the writer's preceding publications (23, 24). It consists of a binomial system in which the first word (light, intermediate, dark) refers to the depth of pigmentation of the mycelium immersed in the agar substratum, and the second (raised, semi-raised, appressed) to the amount of aerial mycelium present. The wild type of each *Fusarium* studied was designated as "light raised" and provided a standard of comparison for the mutants derived from it.

For spore measurements and microphotographs the material under study was mounted in a weak aqueous solution of ruthenium red, following the method employed by Brown and Horne (9).

### Monosporous Isolations from Abnormal Areas

All the *Fusaria* studied here, when grown in culture on potato dextrose agar, produced a loose mycelial type of growth that spread out uniformly from the point of inoculation and bore small conidia evenly and in most cases abundantly with no evidence of localization. In the *Elegans Fusaria* the uniformity of the mycelial growth was commonly disturbed by two main types of cultural phenomena to which the terms 'patch mutant' and 'sclerotium' have been variously applied. These will be considered in turn.

#### (A) Patch Mutants

In the present studies the purpose of the first part of the experimental work was to determine whether areas similar in appearance and in origin to the patch mutants of the muskmelon *Fusarium* occurred in other members of the genus. In this connection Petri dishes poured with 15 cc. of potato dextrose agar as well as test tube slants prepared with the same medium were inoculated from the soil tube stock cultures or with single spores of the organisms in question. The resulting growth in each case was carefully scrutinized for the appearance of abnormal areas in the otherwise uniform mycelium. These sometimes appeared early, becoming evident within three or four days in cultures originating from a single spore, but usually were not conspicuous until a few weeks had elapsed. Monosporous isolations were made from a large number of such places, and also from adjacent normal-appearing areas, which served as checks, about 20 single spore cultures being obtained in each case. In Table II the results of this work are summarized. It will be noted that in all the organisms tested, a large proportion of the cultures thus obtained from the patch mutants were of a type that differed from the 'parent' form. A total of 86 patch mutants were investigated in this manner, and from 66 of these, mutants were obtained. It is likely that more would have yielded mutants had a greater number of single spore cultures been obtained. In all but four instances only one type of mutant was obtained from a given patch mutant, thereby indicating that each of these had resulted from a single mutation. The patch mutants concerned in the four cases mentioned each yielded two mutant types. Although mutants were obtained in a few cases from normal-appearing areas, the number was comparatively low. The indication is, however, that mutant material is not necessarily confined to visibly abnormal areas of the cultures.

The patch mutants encountered during these studies showed a wide range of morphological variation. The commonest type throughout the series was expressed as a loose, rather diffuse area that first became evident in the aerial mycelium as shown in Fig. 1 (A), and tended to spread until a fairly wide area was covered, as in Fig. 9 (F). Both these illustrations are from the muskmelon *Fusarium*, and two other examples from this organism are shown in Fig. 6. A number of such patch mutants appearing in a slant culture of *F. moniliforme* are shown in Fig. 4. In the latter the patch mutants were rendered very conspicuous by the fact that the aerial mycelium collapsed

TABLE II

SUMMARY OF THE RESULTS OBTAINED BY MAKING MONOSPOROUS OR MONOHYPHAL ISOLATIONS FROM ABNORMAL AREAS APPEARING IN CULTURES OF *Fusarium*

Organism	Patch mutants		Sclerotia		Normal-appearing areas	
	Number tested	% of isolates that were mutants	Number tested	% of isolates that were mutants	Number tested	% of isolates that were mutants
Muskmelon <i>Fusarium</i>	19	46	6	0	11	1.5
Pea <i>Fusarium</i>	6	37	13	1.5	6	0
Soybean <i>Fusarium</i>	10	10	20	0	17	0.4
Tomato <i>Fusarium</i>	5	54	—	—	3	0
Aster <i>Fusarium</i>	3	11	—	—	1	0
Navy bean <i>Fusarium</i>	6	19	—	—	4	0
Gladiolus <i>Fusarium</i>	6	19	—	—	2	0
Cabbage <i>Fusarium</i>	9	21	—	—	4	1.1
Flax <i>Fusarium</i>	4	41	—	—	4	1.4
Cotton <i>Fusarium</i>	2	39	—	—	2	0
Cowpea <i>Fusarium</i>	2	45	—	—	1	0
Soil <i>Fusarium</i> A	5	60	4	0	4	0
Soil <i>Fusarium</i> B	4	49	—	—	2	0
<i>F. moniliforme</i>	2	52	—	—	1	0
<i>Gibberella Saubinetii</i>	2	77	—	—	1	0
<i>F. Scirpi</i>	1	100	—	—	1	0
Totals	86		43		64	

elsewhere in the culture. Fig. 5 shows the result of making monosporous isolations from the largest of the patch mutants in Fig. 4. It will be noted that of the 23 cultures on the plate, five are mutants with abundant aerial mycelium. Similar isolations from the appressed area of the slant shown in Fig. 4 yielded all wild type cultures. In Fig. 7 is shown a patch mutant of the flax *Fusarium* photographed from beneath; a distinct darkening effect is noted, which is rather unusual, since most of the loose type in this organism as well as in others had no marked effect on pigmentation. The result of making monosporous isolations from this patch mutant is shown in

FIG. 1. Slant culture of the muskmelon *Fusarium* inoculated 21 days previously, showing two patch mutants (A and B). FIG. 2. Appressed mutant culture of muskmelon *Fusarium* obtained by transferring four successive times a culture similar to that shown in Fig. 1. FIG. 3. Large patch mutant and a number of smaller ones appearing in a month-old slant culture of Soil *Fusarium* A. FIG. 4. Numerous patch mutants in a month-old slant culture of *F. moniliforme* Sheldon. FIG. 5. Plate of monosporous cultures obtained from the large patch mutant near centre of slant culture shown in Fig. 4. The five white cultures are mutant and the remainder wild type. FIG. 6. Two patch mutants appearing in a Petri dish culture of the muskmelon *Fusarium*. FIG. 7. Patch mutant in similar culture of the flax *Fusarium*, as viewed from below. FIG. 8. Twenty-day-old monosporous cultures of the muskmelon *Fusarium* showing four plectenchymatic patch mutants (A, B, C, D) in an early stage of development. FIG. 9. The cultures in Fig. 8 photographed 25 days later, showing advanced stage of development. During the interim two patch mutants (E, F) have become evident. FIG. 10. Same as Fig. 9 but photographed by transmitted light. The dark area to the right of D indicates presence of another patch mutant not visible in Fig. 9.

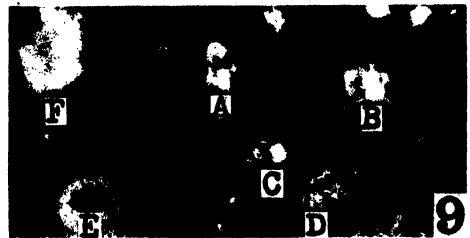
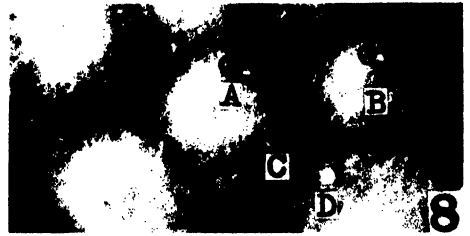
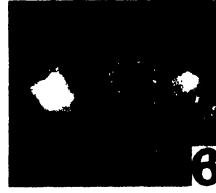
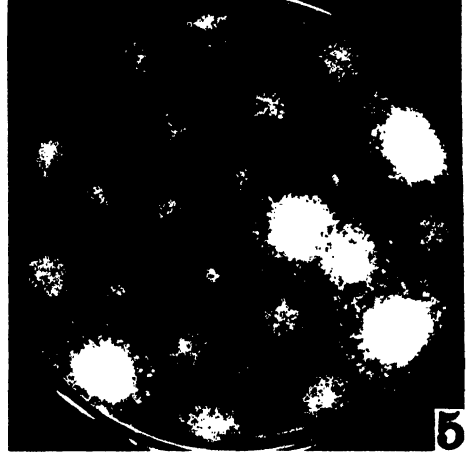
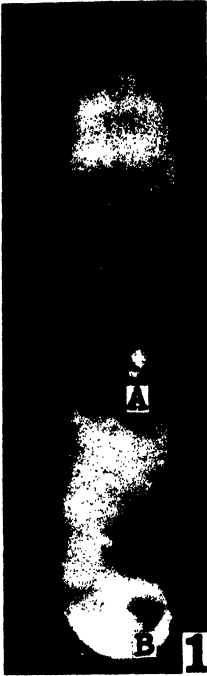




Fig. 22. Loose mound-like patch mutants were sometimes so numerous in Petri dish cultures of the flax *Fusarium* that they made contact with each other all over the surface, concealing the mycelium of the parent. A somewhat similar effect was noted with *Gibberella Saubinetii*, but here the patch mutants were not nearly as abundant. They were likewise prominent in the pea *Fusarium*, and tended to be much larger, one being noted that had achieved a diameter of 10 mm. The tomato *Fusarium* provided another interesting variation of this type of patch mutant, since, although fairly large (up to 5 mm. in diameter) they tended to be very inconspicuous. Transmitted light was helpful in locating these since the patch mutants were denser than the surrounding culture.

The other main type of patch mutant was characterized by the presence of a certain amount of 'plectenchyma,' composed of swollen mycelial cells with thick walls. Razor sections of plectenchyma had a distinctly cellular appearance and the term "Plectenchyme" applied by Appel and Wollenweber (2) to similar structures that they found in cultures of *Fusarium* seems appropriate. Patch mutants containing plectenchyma may be separated into two groups: (1) those in which a dense growth of aerial mycelium is associated with the plectenchyma, and (2) those composed almost exclusively of plectenchyma. The first group has been observed to appear consistently in cultures of the muskmelon *Fusarium*. Figs. 1 (B) and 9 (E) provide examples. These patch mutants tend to be deeply pigmented as viewed from beneath, and this dark appearance usually becomes evident from above later on, showing through the aerial mycelium, which eventually disappears over the area. On occasion the darkening effect may be almost completely absent and the structures assume a light brownish colour. Among the other *Fusaria* studied, the patch mutants of the cabbage pathogen resembled this type rather closely, but the darkening effect did not occur. The other organisms rarely developed this type of patch mutant, but an example is shown in Fig. 3. Here, however, plectenchyma development was slight. The second group mentioned above was widespread among the *Elegans Fusaria*, only the pea, cabbage, and flax *Fusaria* failing to develop them conspicuously, and they were especially prominent and abundant in the muskmelon, aster, navy bean, and gladiolus *Fusaria*. These bodies first become evident as droplets of clear liquid resting on the aerial mycelium, as shown in Fig. 8. It would perhaps be more correct to regard the droplets as hanging downward from the aerial mycelium since the Petri dishes were always kept inverted. About a week later a light brownish body is noted beneath the drop and it gradually increases in size, attaining a diameter up to 4 mm. or more. This development is illustrated in Figs. 8 and 9, the time interval being 25 days, during which the bodies became greatly enlarged and the drops finally disappeared. It is apparent from Fig. 10 in which transmitted light was employed that they are dense in structure and are sharply distinct from the surrounding mycelium. The body labelled C in Fig. 10 presents the appearance of spreading from a central point of origin. Razor sections of these bodies examined micro-



scopically revealed them to consist of a thick rind-like wall about 100 to 300 $\mu$  in thickness enclosing a central hollow usually containing some loose hyphal material. These features are illustrated in Fig. 18, which shows a longitudinal section of one of the bodies from a culture of the gladiolus *Fusarium*. Knotted or bladder-shaped bodies possessing a tissue-like or cellular structure have been described in cultures of *Fusarium* by Appel and Wollenweber (2), and these would seem to correspond to this type of patch mutant.

An unusual type of patch mutant that cannot be grouped with any of the preceding was noted in *F. Scirpi*. It took the form of a soft, mound-like body about 3 mm. in diameter that was found to be composed almost entirely of macroconidia, with no plectenchyma and very little mycelium.

In addition to the differences in general appearance and in compactness, the patch mutants frequently differed from the surrounding normal areas of the cultures in regard to type and abundance of sporulation. Chlamydo-spores were usually very abundant in patch mutants; in fact in the tomato *Fusarium* the presence of these spores was an aid in locating the relatively inconspicuous patch mutants found in this organism. Certain of the patch mutants were found to produce large numbers of long, multiseptate spores.\* This kind of patch mutant was definitely in the minority, comprising not more than 10 to 20% of the total, and these showed no consistent morphological differences from those that bore few or no macroconidia. For example, although only the larger of the two patch mutants shown in Fig. 6 bore macroconidia, there was no obvious morphological difference between them other than size, which elsewhere was not found correlated with macroconidial production.

Efforts to correlate the types of patch mutant with the types of mutant they yielded were not consistently successful. In Fig. 1, for instance, although the two patch mutants are quite dissimilar in appearance, the same type of mutant (light appressed) was obtained from each. The two patch mutants shown in Fig. 6 appear to be of the same general type but the mutants isolated from them differed. A dark semiraised mutant was obtained from the larger one and a light raised mutant from the other. In Fig. 9 some evidence of correlation was noted since (E) yielded light appressed mutant cultures and (F) light semiraised. The mutants obtained from the patch mutants that consisted almost entirely of plectenchyma were mostly of the light raised type, closely resembling the wild type.

In one respect, however, a definite correlation was established between the nature of the patch mutants and that of the mutant cultures isolated from them. Monosporous isolations from patch mutants producing abundant

\* The terms 'macrospore' and 'macroconidium' are commonly applied to *Fusarium* spores of this type in the literature to distinguish them from the smaller nonseptate spores or 'microconidia.' These terms will be employed here with the proviso that the two types of conidia are not regarded as sharply distinct from each other, a view that is in conformity with the opinion expressed by Appel and Wollenweber (2). Macroconidia would appear to be simply large microconidia and this is supported by the observation that in mutants that produce both macro- and microconidia it is usually possible to find all gradations of size and shape between the two extremes. The same is true of the wild types under conditions in which they produce macroconidia as well as microconidia.

macroconidia usually yielded mutants that likewise produced abundant macroconidia. But in a few exceptional instances the mutants obtained from patch mutants producing a small proportion of macroconidia (up to 10%) failed to produce anything but microconidia when grown in the pure state on the same medium. Since the wild types also produced only microconidia, it is evident that in some manner the mixture of the two strains led to the production of macroconidia. Chlamydospores, as stated above, were generally abundant in the patch mutants, and most of the mutants obtained from the latter produced this type of spore more abundantly than did the wild type. It should be noted here that the quantity of chlamydospores and the proportion of macroconidia to microconidia tended to increase as the cultures aged.

Since patch mutants are thus shown to be quite common in cultures of a variety of *Fusaria*, one would expect to find structures of similar origin mentioned frequently in the voluminous literature on the genus. This, however, is not the case. References to 'sporodochia,' on the other hand, are very numerous, but in the cultures studied here no structures were ever observed that, in the writer's opinion, could properly be termed sporodochia. These two prominent discrepancies could readily be resolved were it established that the 'sporodochia' are actually patch mutants. In this connection it may be pointed out that the patch mutants bear a strong similarity to the 'sporodochia' of previous writers in two important respects: (a) in morphology, and (b) in cultural behaviour. To consider the former, Appel and Wollenweber (2) state in regard to sporodochia: "Wo Sporodochien entstehen, können diese aus einem lockeren fädigen Hyphengeflecht hervorkommen oder aus mehr oder weniger begrenzten Plectenchymknäueln." These two types of sporodochia correspond very closely to the two main types of patch mutants described above. Sherbakoff (30) regards *Fusarium* sporodochia as being with or without a plectenchymatic base and without any differentiated enclosure. This is also true of the patch mutants. *Fusarium* sporodochia are regarded as a useful source of macroconidia for taxonomic purposes. As Wollenweber *et al.* (44) state: "If we realize the fact that tubercular sporodochia with normal and uniform conidia occur in the majority of *Fusaria* that can be easily grown in pure culture, there will be no difficulty in judging the normal stages and, consequently, in identifying most of these fungi." In the present studies macroconidia were often found to be very abundant in patch mutants. Regarding (b) above, transfers from patch mutants were found, as expected, to give rise to mixed cultures of parent and mutant in which the mutant had a strong tendency to predominate. Certain statements by other writers give clear indication of similar genetic differences between the 'sporodochia' and the surrounding mycelium. In speaking of the plectenchymatic structures that they regard as a form of sporodochium, Appel and Wollenweber (2) state: "Eigentümlich war die Tatsache, dass die Plectenchymentwicklung ebenso wie die der Konidien und des Mycels potenziert werden konnte, wenn man Teile derselben Wuchsform impfte." Wollenweber *et al.* (44) recommend that for the production of abundant spores, transfers should be made from

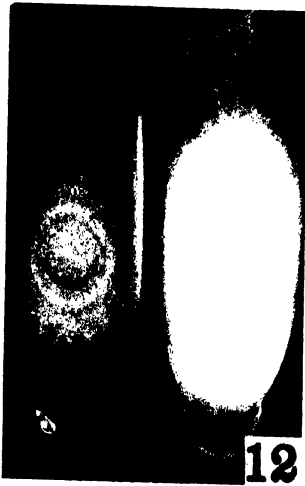
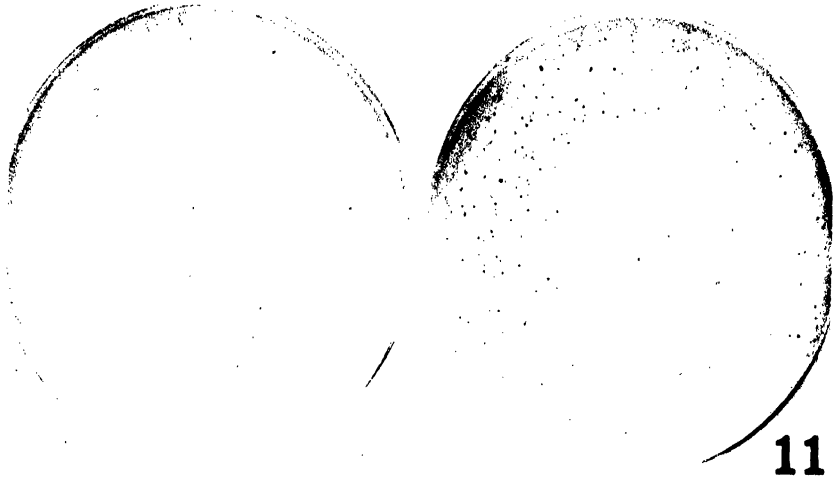
"sporodochia or pionnotes." Sherbakoff (30), in his description of *F. sclerotoides* makes the following statement: "Cultures of this organism on all media may for a long time produce microconidia almost exclusively; but if mature macroconidia from occasionally produced sporodochia are planted, macroconidia may be produced again in new cultures in great abundance and on almost any medium." More recently McLauchlin (22) in regard to a *Fusarium* attacking *Cereus schottii* states: "In the first cultures examined, non-septate spores were produced almost exclusively on all media. When septate spores were transferred from sporodochia, which occurred occasionally, cultures producing septate spores were obtained." There thus seems little doubt that the 'sporodochia' described by these authors as occurring in cultures of *Fusarium* were actually patch mutants.

The importance of this conclusion to *Fusarium* taxonomy is obvious since measurements of "macroconidia from sporodochia" are frequently encountered in descriptions of species of *Fusarium*. If these structures are the result of mutations occurring in the cultures studied, then it is reasonable to question whether such measurements can be of reliable diagnostic value. Since a given patch mutant may have resulted from the appearance of any one of a great variety of mutants, it seems over-optimistic to hope that conidia obtained from such structures would be sufficiently constant to serve for identification. This point will be considered more fully in the second paper of the present series.

### (B) *Sclerotia*

In descriptions of *Elegans Fusaria* references to dark blue or bluish-green sclerotia are often encountered. In the present studies structures corresponding to these appeared frequently in cultures of the muskmelon, pea, and soybean *Fusaria*, and in Soil *Fusarium A*, but were very few or absent in the others. Sometimes these were very abundant, as many as 200 or more appearing in a single Petri dish culture, and they differed from any of the patch mutants already described in that, as a rule, they were much smaller (1 mm. in diameter or less) and were dark bluish-green in colour. In the earlier stages of development they were usually light brown. In common with the plectenchymatic type of patch mutant illustrated in Fig. 18, the sclerotia were found to consist of a dense wall surrounding a less compact central region,

FIG. 11. Induced sclerotia in pea *Fusarium*, as viewed through bottom of Petri dish. Left, check plate inoculated with a spore suspension of the wild type before the agar solidified. Right, same as check but conidia and mycelium of the mutant shown in Fig. 12 were added to the wild type spore suspension before inoculating. Photograph taken 20 days later. FIG. 12. Six-day-old slant cultures of wild type (right) and mutant isolated from sclerotium of the pea *Fusarium*. FIGS. 13 and 14. Razor sections of two sclerotia from 37-day-old single spore cultures of the pea *Fusarium*.  $\times 75$ . FIG. 15. More detailed view of central region of the section shown in Fig. 14, indicating cellular structure.  $\times 330$ . FIGS. 16 and 17. Razor sections of two induced sclerotia from 15-day-old mixed culture prepared at same time and in same manner as that shown in Fig. 11.  $\times 75$ . FIG. 18. Longitudinal section of plectenchymatic patch mutant of *gladiolus Fusarium* from 15-day-old single spore culture.  $\times 75$ .





and these features are evident in the sclerotia of the pea *Fusarium* shown in Figs. 13 and 14. Fig. 15 shows the plectenchymatic structure in more detail, indicating that the bodies consist of densely compacted, thick-walled hyphae of varying width.

The structural similarity of the sclerotia to certain types of patch mutant suggested that a connection might likewise exist between the sclerotia and the incidence of mutations. This was investigated in some detail by making 'biscuit-cutter' isolations from 43 of these, obtained from cultures of the muskmelon, pea, and soybean *Fusaria*, and Soil *Fusarium* A. The sclerotia concerned were crushed in drops of distilled water on slides to obtain suspensions of conidia and mycelial fragments that were streaked on agar plates. The results are listed in Table II and, as shown, only in the case of the pea *Fusarium* were mutants obtained. From two sclerotia produced by this organism slow-growing mutants were isolated. One of these consisted mainly of immersed brownish mycelium that produced extremely abundant macroconidia and these covered the cultures in a thick slimy layer. This mutant is illustrated in Fig. 12. It was found possible to reproduce sclerotia experimentally by preparing spore suspensions of the mutant and of the wild type and mixing a few drops of each in Petri dishes containing 15 cc. of potato dextrose agar just before the latter solidified. About 10 days later sclerotia began to appear in the plates containing the mixture whereas in plates containing the wild type alone they did not become evident until at least two weeks after inoculation and even then were much less abundant. The results of this experiment are illustrated in Fig. 11. Razor sections of the 'induced' sclerotia are shown in Figs. 16 and 17, and it is apparent that structurally these are quite similar to those in Figs. 13 and 14, which appeared spontaneously in wild type cultures.

The foregoing is strong evidence for the view that these sclerotia are of mutant origin. However, the failure to obtain mutants from the many other sclerotia studied in this manner vitiates the conclusion to some extent. If *Fusarium* sclerotia always result from mutations, it should be possible to isolate mutant material from them more readily. Frequently the point of origin of a sclerotium is seen to be a small piece of debris in the agar. Possibly, therefore, a stimulus is required to initiate sclerotial development, and mutation may represent one such stimulus.

Since the *Fusaria* are commonly regarded as imperfect stages of Ascomycetes, the general appearance of the razor sections could be taken as suggesting that the sclerotia are actually abortive perithecia. No evidence of asci or ascospores was found in the crushed mounts and sections that were studied.

It is not implied that sclerotia, and likewise sporodochia, wherever they appear, may be of mutant origin. It must be emphasized at this point that reference is made here only to structures arising in cultures of *Fusarium*.

## Cultural Degradation

In a preceding publication (23) the phenomenon termed 'cultural degradation' was discussed. The term was employed to designate the process by which the wild type of the muskmelon *Fusarium* was displaced in culture by mutants. An example is provided by Figs. 1 and 2. The former shows a slant inoculated with a purified culture of the wild type and the latter a similar culture that was transferred four times during a period of three months subsequent to inoculation, as a result of which the original 'raised' character of the culture was lost and the immersed mycelium of an appressed mutant type became predominant, as illustrated.\* The term 'deterioration' has been applied by Lucas (20) to a similar phenomenon in *Colletotrichum falcatum* Went. in which "patch variants" appeared and tended to displace the original type. The experimental work described previously (23) indicated that the process in the muskmelon *Fusarium* is accelerated by frequent transferring, certain media favouring the loss of the wild type more than others. In tubes of sterilized soil and on slants of soil agar, even when the latter were transferred, the wild type remained pure. The evidence suggested that this was not because the mutants were unable to displace the wild type on this medium but rather that under such conditions of culture the organism was genetically very stable.

To determine whether this situation is more widespread in the genus *Fusarium*, an experiment was performed involving 10 *Fusaria* under four cultural conditions: (1) potato dextrose agar slants, transferred; (2) soil agar slants, transferred; (3) test-tubes of sterilized soil, transferred; (4) test-tubes of sterilized soil, not transferred. In preparing the soil tubes, sandy loam soil was sifted through a fine screen and added to test-tubes to within an inch of the top. To ensure a sufficient content of moisture about 2 cc. of water was added to each before they were plugged and sterilized. The slants and tubes were inoculated with freshly isolated single spore cultures of the *Fusarium* wild type to be studied and were transferred successively during a period of three months except for the set of soil tubes that was not transferred. There were three replicates of each combination of organism and cultural treatment. After the third transfer monospore analyses were made of the contents of the tubes in the first replicate by adding sterilized distilled water to each and spreading the resulting suspension, diluted if necessary, over the surface of potato dextrose agar in Petri dishes from which single germinating spores were removed by the 'biscuit-cutter' technique. In the soil tubes, very few spores were produced and monohyphal isolates were

\* This would appear to correspond to the "pionnotal" form of growth referred to by Wollenweber (42) as a stage with a maximum of conidia and a minimum of aerial mycelium. All the *Fusaria* studied here, when isolated from nature, produced on potato dextrose agar a form of growth with abundant aerial mycelium. But cultures of *F. udum* Butler isolated by the writer from material of Cajanus received through courtesy of Dr. B. B. Mundkur, New Delhi, India, showed a strongly "pionnotal" form of growth with abundant conidia and no aerial mycelium. It is evident that, by transferring, the initial growth form of the muskmelon *Fusarium* was changed to a type of growth resembling *F. udum*, and a danger of taxonomic confusion is apparent, if such transferring is not avoided.

TABLE III

COMPARISON OF FOUR CULTURAL METHODS REGARDING THEIR EFFECT  
ON THE STABILITY OF 10 *FUSARIA*

Organism	Percentage of mutants isolated via 'biscuit cutter' technique											
	Potato dextrose agar slants			Soil agar slants			Sterilized soil in test tubes					
	Transferred			Transferred			Transferred			Not transferred		
	(1)*	(2)*	(3)*	(1)*	(2)*	(3)*	(1)*	(2)*	(3)*	(1)	(2)	(3)
Muskmelon <i>Fusarium</i>	100	100	100	0	100	0	0	0	—	0	0	0
Soybean <i>Fusarium</i>	0	100	100	4.5	0	0	0	0	0	0	0	0
Tomato <i>Fusarium</i>	85	100	100	27	18	96	0	0	0	0	0	0
Aster <i>Fusarium</i>	59	100	100	100	4.5	0	0	0	0	0	0	0
Navy bean <i>Fusarium</i>	72	100	100	0	0	0	0	0	0	0	0	0
Flax <i>Fusarium</i>	100	100	100	100	100	37	0	0	15	0	0	9
Cotton <i>Fusarium</i>	100	100	100	0	0	100	0	0	0	4	0	0
Soil <i>Fusarium A</i>	100	100	100	0	0	0	0	0	0	0	0	0
Soil <i>Fusarium B</i>	100	100	100	0	27	0	0	0	0	0	0	0
<i>F. moniliforme</i>	100	100	100	100	100	100	0	0	0	0	0	0

(1)\* Transferred three times.

(2)\* Transferred four times.

(3)\* Transferred five times.

made by the same technique from hyphae that grew out of small grains of soil scattered sparsely over the surface of the agar. The same was done with the second replicate after the fourth transfer and after five transfers the third replicate was analysed. From each tube or slant a total of 22 'biscuit-cutter' isolations was attempted, a few of which usually failed to develop, and the resulting cultures, 2340 in all, were examined in search of mutants. The results thus obtained are summarized in Table IV.

In general the other nine *Fusaria* resembled the muskmelon *Fusarium* as regards degree of stability under the four types of cultural treatment. The wild types were not displaced as frequently on soil agar as on potato dextrose agar even though in the former medium many instances of loss of the wild type were noted. For the first time an instance of the loss of the wild type of the muskmelon *Fusarium* on soil agar slants was observed. In a previous publication (23) two experiments were described that included seven instances in which soil agar slants were inoculated with purified wild type cultures and transferred successively but in none of these was the wild type lost. Although the muskmelon *Fusarium* may be fairly stable on soil agar, this does not appear true for certain others, notably the tomato, aster, and flax *Fusaria*, and *F. moniliforme*. The soil tubes were definitely the most favourable medium for the maintenance of the wild types although even in these there was some indication of instability in the flax and cotton *Fusaria*, three tubes out of 12 yielding a low proportion of mutant cultures. The number of mutants thus obtained from soil tubes is too low for any conclusions to be



TABLE IV

COMPARISON, WITH RESPECT TO THE NUMBER OF MUTANT CULTURES PRODUCED, OF SLOWLY AND RAPIDLY GERMINATING SPORES\* OBTAINED FROM PATCH MUTANTS OF NINE *FUSARIA*

Organism	Number of patch mutants involved	Slowly germinating spores		Rapidly germinating spores	
		Number of cultures obtained	Number that were mutants	Number of cultures obtained	Number that were mutants
Muskmelon <i>Fusarium</i>	5	61	35	62	43
Pea <i>Fusarium</i>	4	38	6	47	27
Soybean <i>Fusarium</i>	1	10	3	12	6
Tomato <i>Fusarium</i>	3	29	16	32	26
Aster <i>Fusarium</i>	1	12	0	11	7
Gladiolus <i>Fusarium</i>	1	8	0	13	3
Soil <i>Fusarium</i> A	1	9	2	5	4
Soil <i>Fusarium</i> B	2	20	5	18	15
<i>F. moniliforme</i>	1	10	0	13	5
Totals	19	197	67	213	136

\* Term includes both chlamydospores and conidia.

drawn regarding the effect of transferring on the maintenance of the wild type under these conditions, but it would be reasonable to expect that successive transferring to fresh, moist tubes would give any mutants that did appear the opportunity to multiply, whereas this could hardly take place to advantage in a tube allowed to become dry and not transferred. Orton (26, 27) cultured *F. niveum* E.F.S. in flasks of sterilized soil and at monthly intervals removed samples of inoculum. In one experiment 41 out of 8220 differed from the parent culture.

The mutants obtained during this experiment were in general similar to those derived from patch mutants by monosporous isolations, lending support to the view that the degradation process is initiated by local mutations. The slant shown in Fig. 1 had been inoculated 21 days previously with a purified wild type culture of the muskmelon *Fusarium* in connection with the experiment just described. During this period two patch mutants (A and B) became evident and monosporous isolations from both of these yielded cultures of a light appressed mutant but similar isolations from a point midway between them yielded the wild type only. When, after three transfers, the spore suspension made from the contents of this tube was analysed, 20 out of 21 single spore cultures were of the light appressed type, which is strongly indicative of a connection between the patch mutants and the degradation process. A light semiraised mutant culture was also obtained, which is explicable on the basis of an additional mutation.

It is natural to wonder how a very small number of mutations, each arising presumably in a single cell of the mycelium, can eventually succeed in dominating the parent type or in displacing the latter completely. This problem

was discussed to some extent in a preceding publication (23) in which it was pointed out that the mycelium of certain mutant types of the muskmelon *Fusarium* was able to invade the areas occupied by the wild type along the line of contact, a phenomenon termed "cultural interaction." Monosporous isolations from this invaded zone yielded a much larger proportion of mutant cultures than of the wild type, indicating that the former was succeeding in dominating the parent in this region. The same process was assumed to account for the increase in size of the patch mutants. Transferring favoured the loss of the wild type perhaps because each transfer mixed the inoculum and as new growth resulted from this, the mutant would have additional opportunity to dominate adjacent wild type areas. In addition to this method of securing the ascendancy over the parent type, evidence was obtained of a further method in the observation that, when monosporous isolations were made from patch mutants of the muskmelon *Fusarium*, the spores that germinated rapidly included a much higher proportion of mutants than those that germinated somewhat slowly. The conclusion seemed reasonable that when inoculum containing spores of both mutant and wild type is transferred, this rapidity of germination would give the former the advantage. During the present studies when monosporous isolations were made from patch mutants, spores that germinated relatively rapidly were kept separate from the slower ones in some instances and the number of mutant cultures in each was compared. The results, which are summarized in Table IV, indicate a correlation between the rapidity of germination of the spores and the number of mutants included, since from a total of 19 patch mutants 34% of the spores that germinated slowly produced mutant cultures whereas of those that germinated relatively rapidly 64% were mutants. Moreover, the table does not consider the case noted in *F. Scirpi* in which all of the spores obtained from a patch mutant germinated rapidly and gave rise to mutant cultures, as compared with those from an adjacent normal-appearing area, all of which germinated relatively slowly and produced the wild type.\* Exceptions were provided by the flax and cabbage *Fusaria* in which the reverse seemed true but since only two patch mutants of the flax *Fusarium* and one of the cabbage *Fusarium* were studied in this regard, it is possible that more extensive data from these organisms would bring them into line with those in Table IV. The evidence provided by this table strongly suggests that rapidity of germination is a common occurrence in the spores of mutants derived from *Fusarium* wild types and accounts in part for the ease with which the latter are displaced in artificial culture. The existence of an additional means of accomplishing this end, as evidenced by the fact that

\* Rapidity of germination has not been found correlated with rapid growth rate in pure culture; no mutants were found that grew conspicuously faster than their respective wild types and frequently, as shown in the muskmelon *Fusarium* (23), they grew somewhat less rapidly. But a correlation has been noted between rapidity of germination and spore size, macroconidia usually germinating much more rapidly than microconidia, perhaps owing to the presence of a larger supply of readily available food material, which gives them an initial temporary advantage. Certain of the slow-growing mutants were found to invade adjacent cultures of the wild type producing conspicuous cultural interaction, which suggests that a slow growth rate would not hinder a mutant from dominating the wild type by this method.

patch mutants increase in size owing to the invasion of the surrounding wild type by mutant material, has been pointed out above, and it seems logical to conclude that these two sets of observations account for the difficulty experienced in maintaining pure cultures of *Fusarium* wild types by the usual laboratory method.

The 10 *Fusaria* employed in the cultural degradation experiment yielded mutants that were of the same general types. Appressed mutants were the most common but semiraised and raised types were also found. In the potato dextrose agar slants the proportions were 34 appressed: 5 semiraised: 4 raised. The number of times that raised mutants were isolated from soil agar slants was higher, the proportions being 5 appressed: 1 semiraised: 7 raised. The indication is that the nature of the medium in some manner affects the type of mutant arising in it.

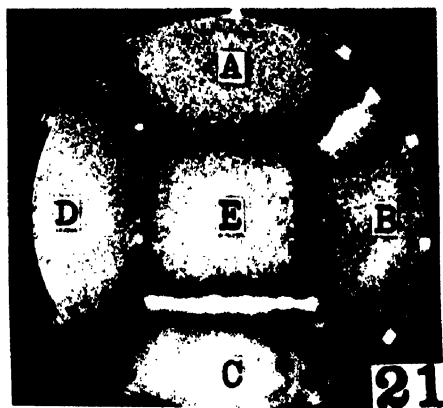
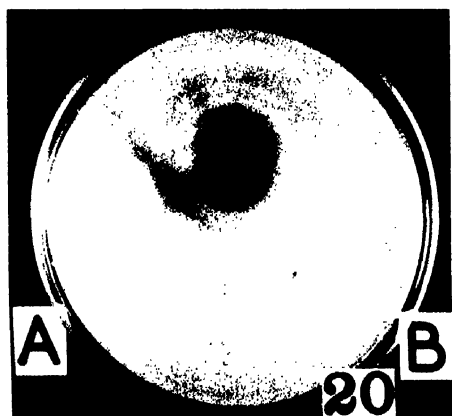
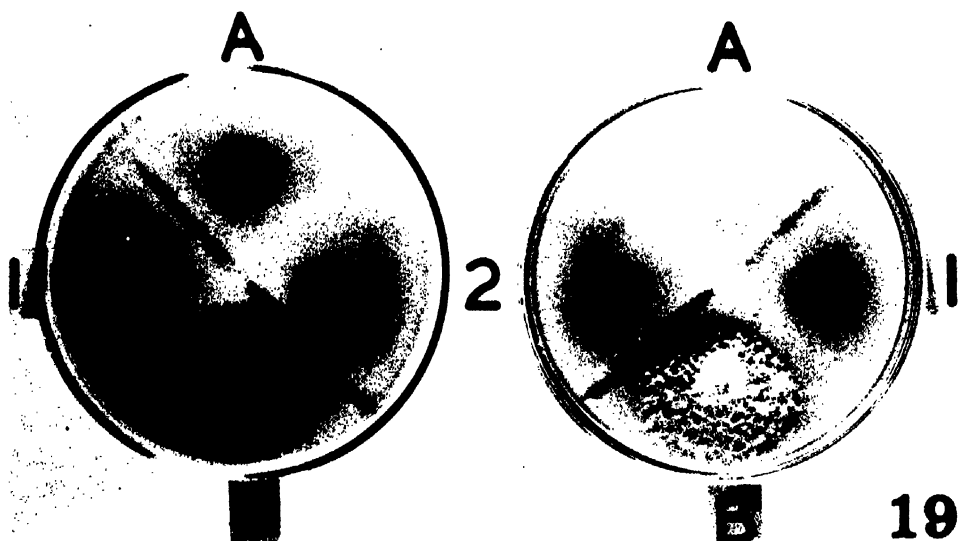
Macroconidia were not always abundant in the transferred slants but sometimes became very numerous, comprising up to 20% of the conidia present, and certain of the mutants obtained from the slants bore numerous and conspicuous macroconidia. These observations on spore production are of taxonomic interest. Wollenweber *et al.* (44) recommend transfers to obtain "normal" conidia for taxonomic purposes. Macroconidia, regarded by them as the "normal" type, have been obtained here by repeatedly transferring purified wild type cultures on potato dextrose agar. Since the *Elegans* wild types produced no macroconidia (or at least very few) on this medium and since monosporous isolations from the transferred slants consistently yielded mutant cultures many of which bore macroconidia, the conclusion seems evident that transferring favours macrospore production because it leads to the displacing of the wild types by such mutants. It would seem inadvisable to employ such macroconidia in taxonomic studies, a point that will be considered more fully in a subsequent publication.

### Cultural Interaction

The phenomenon of cultural interaction has previously been described (23) and its possible value as a taxonomic criterion was indicated. These investigations have been carried further employing a number of *Fusaria* in an effort to assess the extent to which the phenomenon may be employed to identify *Fusarium* wild types.

Definite interaction was observed in most of the *Fusaria* studied but the intensity of the effect varied greatly. The most striking examples of inter-

FIG. 19. Cultural interaction between two *Fusarium* wild types and their derived mutants. A = cotton *Fusarium*. B = cowpea *Fusarium*. 1 = light appressed mutant from A. 2 = light appressed mutant from B. The plate to the left is inverted. FIG. 20. Cultural interaction between two wild types and a mutant. A = Soil *Fusarium* A. B = Soil *Fusarium* B. Above is a dark appressed mutant derived from A. The plate is inverted. FIG. 21. Cultural interaction between isolates of the flax *Fusarium*. FIG. 22. Cultural interaction in the flax *Fusarium* between 5-day-old monosporous cultures of the wild type and a light semiraised mutant, all isolated from the patch mutant shown in Fig. 7. FIG. 23. Appearance two weeks later of the plate shown in Fig. 22.





action were noted in the muskmelon, cabbage, flax, cotton, and cowpea Fusaria. Interaction was apparent but not usually conspicuous in the pea, soybean, and tomato Fusaria, in Soil Fusarium *A*, Soil Fusarium *B*, and in *F. moniliforme* and *F. Scirpi*. No evidence of the phenomenon was noted in the aster and navy bean Fusaria or in *Gibberella Saubinetii*. The intensity of the interaction in those Fusaria that displayed it varied greatly with different mutants, a point that has previously been pointed out in the case of the muskmelon Fusarium (23).

The possible value of interaction as a taxonomic criterion was tested by allowing mutants that gave pronounced interaction with their parent cultures to contact other wild types growing in the same Petri dishes. Fig. 19 shows an instance in which light appressed mutants of the cotton and cowpea Fusaria are matched with their respective wild types. The specificity displayed here is very marked, the interaction expressing itself as a dark band in the immersed mycelium between each wild type and its derived mutant but there is no suggestion of this between the cowpea wild type and the cotton mutant and vice versa. Similarly, in Fig. 20 a dark line is evident between Soil Fusarium *A* and a dark appressed mutant isolated from it but this is not evident between the mutant and Soil Fusarium *B*. Two additional experiments not illustrated here add further evidence for specificity. (1). In the flax Fusarium, cultural interaction was not noted between a light appressed mutant and the wild types of the muskmelon Fusarium, Soil Fusarium *A*, and Soil Fusarium *B* but it occurred consistently between this mutant and its parent wild type. (2). In the cabbage Fusarium a mutant that interacted strikingly with the parent failed to give any evidence of this effect when it was allowed to contact the muskmelon, pea, soybean, tomato, aster, navy bean, cotton, and cowpea Fusaria, Soil Fusarium *A* and Soil Fusarium *B*. These experiments taken in conjunction with those outlined in a preceding publication (23) suggest that the phenomenon is sufficiently specific to serve as an aid to taxonomy. It is particularly noteworthy that by this means it was possible to separate the two saprophytic Elegans Fusaria, since differential pathogenicity cannot be employed in such organisms to check a diagnosis.

Interaction is not confined to forms appearing in culture. In Fig. 21 are shown the results of an experiment in which a number of wild type strains of the flax Fusarium\* were allowed to contact each other. It will be noted that interaction was very marked between *A* and *B*, and *C* and *E*, and served to distinguish all the strains shown with the exception of *A* and *D*. In a separate experiment, *D* was found to interact with *B* much less strongly than did *A*. Thus all five strains shown can be separated by means of cultural interaction.

The expression of interaction between parent and mutant noted in the present studies may be divided into three main types: (1) that in which the predominant effect is the invasion of the wild type culture by the mutant,

\* Where reference is made elsewhere in this publication to the flax Fusarium, an isolate obtained in 1944 is indicated, and this differed in some respects from the strains shown in Fig. 21, which were isolated in 1945.

exemplified by the muskmelon, pea, tomato, and cabbage *Fusaria*; (2) that in which a dark band appears between the two, exemplified by the cotton *Fusarium*, cowpea *Fusarium*, Soil *Fusarium A*, and on occasion by the muskmelon *Fusarium*; (3) that in which the wild type overgrows the mutant culture, the only instance of this type being the flax *Fusarium*. The latter type of interaction is illustrated in Figs. 22 and 23. Fig. 22 shows a plate of monosporous isolations obtained from the patch mutant in Fig. 7 and photographed five days after the isolations were made, at which time cultural interaction was just beginning to be evident. Fig. 23 shows the condition of the same plate two weeks later. During the interim the wild type cultures had overgrown more than half of the total area occupied by the mutants. Very little further progress in this regard was made and within 10 days after the photograph for Fig. 23 was taken, the ridge of aerial mycelium had collapsed.

Some workers might be inclined to interpret this marked mycelial growth at the point of contact as an example of increased mycelial vigour resulting from a heterocaryotic state. However, the results of certain investigations performed in this connection lend no support to the view. If a heterocaryotic state existed in this ridge of mycelium, then it would be expected that single hyphae isolated from it would, in a large proportion of cases, give rise to mixed cultures. Accordingly, mycelium was taken from the area in question and was macerated and teased out in order to make a suspension in sterilized distilled water. This was then spread over a plate of agar as in the technique for making monosporous isolations. Single hyphae about 5 to 10 cells long were then isolated via the 'biscuit-cutter' method and transferred to other plates. The cultures that grew from the hyphae were either wild type or mutant and the phenomenon was repeated where these contacted each other. In all, 48 single hyphae were isolated, 38 of which gave rise to wild type cultures and the remainder to the mutant. In no case was a mixed culture obtained and it thus seems unlikely that heterocaryosis can account for the phenomenon.

The explanation should, perhaps, be sought on ecological rather than on cytological grounds. The cultural environment encountered by the wild type of the flax *Fusarium* where it begins to overgrow the area occupied by the mutant would certainly differ from that in which it was growing up till then and it may well favour an intensified proliferation of aerial mycelium, intermingled with hyphae sent up from the mutant. A similar situation in more or less modified form is likely responsible for the other types of interaction described.

### Discussion

#### (A) *The Nature of Variation in Fusarium*

Variation is a common phenomenon in the genus *Fusarium* and the variants described by other workers resemble those obtained here. The organisms seem to vary between a type with abundant aerial growth and a type in which

all the mycelial growth is immersed in the nutrient substrate. Differences in colour and in the amount and type of sporulation are frequent.

Three main theories have been advanced to account for these variations.

The cyclogenic variation theory as proposed by Leonian (17, 18) represents an effort to account for the appearance of different cultural forms by assuming them to be merely phases in the orbit of variation of a species. As evidence for this he points out the manner in which *Fusarium* isolates were found to fluctuate back and forth during transfers. Since no effort was made to purify the inoculum employed in the transfers, it is not surprising that such fluctuations were observed, and rather than establishing the cyclogenic hypothesis the observations may merely indicate that the inoculum was often a mixture of two or more strains, the proportions of which varied with successive transfers.

Similar views have been expressed by Brierley and Goddard. In 1931 Brierley (6) objected to the term 'mutation' as applied to fungi since he considered that at that time the knowledge of the genetics and cytology of fungi was insufficient to justify it. In his opinion most of the variation phenomena were merely selective eliminations or combinative, dissociative, segregative, or cyclogenic changes originating in one form or another of sexual or somatic fusion. He considered hyphal fusions to be more important than we realize, but did not explain how these could induce changes in the progeny of a single, haploid, uninucleate spore. Goddard (13) concluded that there was a definite cycle of growth phases through which the fungus passed. She was able to cause five out of eight variants of *Gibberella Saubinetii* to revert to the parent type by transferring them from potato dextrose agar to Leonian's medium. However, the variants were not purified before this transfer and it seems not at all impossible that the inoculum included some of the parent type, which was able to predominate in the new environment.

If 'cyclogenic variation' were responsible for the appearance of variants in *Fusarium* cultures, then reversion to the parent type should be a common phenomenon, but it must be emphasized in this connection that in the writer's experience once a variant was purified by the monosporous (or monohyphal) method, reversion to the parent type never followed. Wellman and Blaisdell (40) and Wellman (38) obtained some degree of reversion with the tomato wilt *Fusarium*, but Brown (8) found no evidence of reversion in the *Fusarium* with which he worked, and Armstrong *et al.* (3) did not observe reversion in *Fusarium vasinfectum* Atk. It therefore seems advisable to look elsewhere for the cause of variability in *Fusarium*.

Variability in many of the Fungi Imperfecti can be explained, according to Hansen (14), on the basis of what he terms the "dual phenomenon." This assumes variation to be the result of a segregating out of genetically different nuclei in a multinucleate mycelium. In a preceding publication Hansen and Smith (15) reported variations in *Botrytis cinerea* Pers. that they attributed to nuclear segregation. If we assume the "dual phenomenon" operates in a



multinucleate form like *Botrytis*, the theory meets difficulties when efforts are made to apply it to *Fusarium*, since a prerequisite of the theory is that the cells of the organism concerned must be multinucleate. This does not seem to be the case in *Fusarium*. It was shown in the muskmelon *Fusarium* (23) that most of the mycelial cells and the microspores were uninucleate. Dickinson (10) studied two species of *Fusarium* and found that each cell in both the mycelium and the conidia contained one nucleus. It is thus impossible to account for the production of variants among the progeny of a single spore by the segregating out of mixed nuclei, and heterocaryosis can hardly be responsible for the sort of variation found in *Fusarium*. Yet Hansen (14), referring to the work of Leonian (17), makes the statement that the cultural variants shown in certain of Leonian's figures "are all most excellent demonstrations of the dual phenomenon." The observations of the present studies suggest a simpler explanation for the sectoring observed: that the inoculum that produced these cultures was a mixture of two types, one or both of which had originated through mutation in culture.

In attempting to apply the "dual phenomenon" to many of the Fungi Imperfecti, Hansen (14) lists over 30 genera, over half of which when analysed by the single spore method were found to be "dual," i.e. composed of two culturally distinct individuals. An intermediate type was also found, which broke down into the two "homotypes." To support this cytologically he states that the spores of *Botrytis* are multinucleate, those of *Phoma terrestris* Han. binucleate, and those of *Verticillium albo-atrum* R. & B. mainly uninucleate. Again, the spores of *Ascochyta Pisi* Lib. are stated to contain seldom more than seven nuclei. But work by Ludwig (21) has indicated that the sporophores of *Ascochyta Pisi* Lib. contain only one nucleus. Dickinson (10) has shown in *Fusarium* and *Helminthosporium* that all the nuclei in a multinucleate or multicellular spore arise from a single parent nucleus, and this point has been confirmed by the writer with *Fusarium*. Hence in the latter three organisms the carryover of a mixed nuclear condition from a heterocaryotic mycelium would be unlikely, although Hansen concludes that this is a common occurrence in *Ascochyta Pisi* Lib. as well as in many other fungi with multinucleate spores.

It would appear that Hansen has failed to give full consideration to the possibility that the variations observed are susceptible to explanation on the basis of mutations in culture, which may be a result of working first with a form like *Botrytis* rather than with a uninucleate form. It would seem more reasonable to study first forms possessing essentially uninucleate cells and thus obtain a basis for reasoning that may be applied to the more complex situation that could exist in multinucleate forms, rather than vice versa.

The weight of evidence accumulated to date definitely favours the view that mutations are responsible for cultural variation of the sort observed in *Fusarium*. It is difficult to conceive how variants may arise among the progeny of a single, haploid, uninucleate spore unless by mutation. Ullstrup (36) and Eide (12) obtained variants in cultures that arose from single asco-

spores of *Gibberella Saubinetii*. Further evidence is provided by the genetic behaviour shown by certain cultural variants. The work of Dimock (11) and of Hansen and Snyder (16) on *Hypomyces* has shown that variants may be inherited as unit characters. Stakman *et al.* (35) have shown mutability and constancy in haploid lines of *Ustilago Zeae* (Beckm.) Ung. to be due to genetic factors. Mention should also be made of the work of Whitehouse (41) and of Lindegren (19) who have demonstrated crossing-over and mapped chromosomes by employing cultural variants of *Neurospora*. Likewise, Shay and Keitt (29) have shown certain mutants that appeared in cultures of *Venturia inaequalis* (Cke.) Wint. to be inherited as unit characters. Beadle and Tatum (4) describe work on *Neurospora* in which by irradiation of spores with X-rays, ultraviolet radiation, or neutrons, mutants differing in their nutritional requirements were obtained. Genetic studies showed these to differ by single genes from the wild type strains from which they were obtained.

#### (B) Variation in Nature

Most of the *Fusaria* studied by the writer to date, when first isolated from plants infected in naturally infested soil, were of a type that produced abundant aerial mycelium on potato dextrose agar. When these were grown for a time on this medium and perhaps transferred several times it was commonly noted that the original mycelial type was displaced by mutants that formed less aerial mycelium and, occasionally, more and larger spores. The fact that the original isolates were not of the appressed type is sound evidence that this process does not occur in the soil. Two reasons may be advanced to account for this. First, the organisms are genetically more stable in the soil than in artificial culture. Evidence to support this is provided by the experiments on cultural degradation, which showed that far fewer mutations appeared in a nutrient medium approximating that of the soil environment, i.e., in tubes of sterilized soil. Second, if variants of the sort that commonly appear in culture do arise in the field they may be less able to survive the competition of other organisms and hence would tend to be crowded out. In this connection it was shown in the case of the muskmelon *Fusarium* (23) that certain mutants that were almost as virulent as the wild type in sterilized soil were definitely less virulent in unsterilized soil and in the field. When such mutants were introduced into the field along with the wild type it was found that after a year they were negligible as a disease factor but the wild type still caused wilt.

That the original isolates of many *Fusaria* are of a type with abundant aerial mycelium is also evident from the observations of other workers. Weindling (37) made a careful study of 13 monosporous isolates of *Fusarium vasinfectum* Atk. and found that all the original isolates from diseased plants were alike and had abundant aerial mycelium. They were all highly pathogenic and tended to be crowded out by less virulent, more appressed variants. All the isolates of *Gibberella Saubinetii* that Ullstrup (36) obtained from perithecia on diseased plants were similar and variants appeared when these

were subcultured. Brown (8) noted that most of his original isolates of *Fusarium fructigenum* Fr. were of the mycelial type and produced small spores. Appel and Wollenweber (2) in describing the basis for the taxonomy of the genus pointed out that many *Fusaria* as isolated from nature developed aerial mycelium with small, low-septate spores. Wollenweber *et al.* (44), in discussing the classification of *Fusaria*, advise various cultural treatments to obtain a type of growth producing "normal macroconidia" in the case of those species the isolates of which persistently produce only microconidia. The evidence seems conclusive therefore that many, if not most, *Fusaria* occur in nature as types that may be altered by a period of growth in culture.

It is not implied that no variation takes place in the field but this must be relatively infrequent and the new forms that may thus become established tend to resemble the parent, the range of variability evidently being far narrower than that observed in artificial culture. For example, while all the initial isolates of the muskmelon *Fusarium* were identical, this did not hold true for the flax *Fusarium*. Over 200 single-spore isolates were obtained in 1945 from surface sporulation on 25 flax plants, and while all the isolates from each plant were usually identical, some cultural differences were noted between those from different plants, at least five types being distinguishable (Fig. 21). Borlaug (5) found a large number of cultural races of *F. Lini* attacking flax in Minnesota. Nelson *et al.* (25) described two forms of *Fusarium Apii* Nelson and Sherbakoff as causing celery yellows in Michigan. Both forms had abundant aerial mycelium and could be distinguished with difficulty in culture. Symptoms characteristic of each were recognizable in the field and often only one form was present in a region where the disease had recently been introduced. Thus it is evident that more than one wild type (or form) may occur in the field. The additional possibility that a number of pathogenic forms may be included should also be considered. Broadfoot (7) found nine forms of *F. Lini* among isolates from various localities. These were very difficult to distinguish in culture but they could be separated by their relative virulence on four flax varieties. Likewise, Borlaug (5) found a number of pathogenic races among his isolates of *F. Lini*, and showed them to be of importance under field conditions. Alexander and Tucker (1) isolated a race of the tomato wilt *Fusarium* in Ohio to which varieties hitherto resistant were found to be completely susceptible. The bearing of the wild type concept on the problem of breeding for resistance has been discussed by the writer in Part II of *Studies on the Fusarium of Muskmelon wilt* (24).

Views differing from those outlined above have been expressed by certain other authors. Sleeth (31) observed a wide range of variation among isolates of the watermelon wilt *Fusarium* from various parts of the United States and assumed that these occurred naturally. A critical examination of the cultural methods he employed casts doubt upon this assertion. For example, some of the isolates were cultured for several years and no mention is made of any effort to maintain them in a pure state. In view of the observations

described here with certain other wilt *Fusaria*, it would be remarkable indeed if during this time they had not changed. Likewise, Wellman and Blaisdell (39) compared 30 isolates of the tomato wilt *Fusarium*, three of which had been in culture prior to 1924, and most of the remainder for at least two years. As would be expected, marked differences both in cultural appearance and in virulence were found among the cultures, but the assumption that these existed as such in the field seems unwarranted. Snyder (32) found similar wide variation among isolates of the pea wilt *Fusarium* and stated the original isolates to show as wide a range of variability as that derived in culture from the progeny of a single spore. Here again the failure to compare the cultures immediately after isolation or to adopt measures to prevent the original cultures from being displaced by mutants vitiates the conclusion that this sort of variation occurs in the field. Both Wellman and Blaisdell, and Snyder, noted differences in virulence among their cultures but no host specialization was found. Armstrong *et al.* (3) found marked differences in pathogenicity and cultural characteristics between isolates of *Fusarium vasinfectum* Atk. from different localities but these authors recognize the fact that the weakly pathogenic, appressed cultures may have originated during the lengthy period in culture. Fresh isolations from diseased plants in the field were characterized by abundant aerial mycelium.

It cannot be overemphasized that in studies concerning the variability of *Fusaria* in nature, cultural comparisons should be made as soon as possible after isolation unless particular care is taken to maintain the purity of the originals. If differences in cultural appearance or host specialization (or both) are noted among the original isolates, these should be regarded as separate wild types of the organism concerned. Mutant strains that may arise when the wild types are cultured on most laboratory media need not be considered seriously either in pathogenicity studies or in the taxonomy of *Fusarium*.

### (C) *The Relation of Variation to Taxonomy*

The importance of the preceding conclusion to *Fusarium* taxonomy is obvious. If a consistent morphological treatment of this difficult group of fungi is to be achieved, care must be taken that the features studied and compared are those of the wild types.

In this connection the problem of maintaining stock cultures is of prime importance and it is felt that the experiments on cultural degradation described in the present studies and in the preceding work on the muskmelon *Fusarium* (23) will provide an answer. The observation was recorded that the wild types were readily maintained in tubes of sterilized soil with little danger of being displaced by mutants, which so frequently happened in potato dextrose agar and to a lesser extent in soil agar. These soil tubes were found to provide a very convenient source of inoculum, wild type cultures being obtainable at will by transferring a few grains of dry soil to the agar medium.

It will not be out of place to describe at this point an experiment involving certain soil tube cultures of the muskmelon wilt *Fusarium*. In August, 1942, eight tubes, which included four soil types, were inoculated with a recently isolated culture of the organism. For a month they were kept in a moist atmosphere and were then allowed to become dry. In August, 1945, transfers of soil grains to potato dextrose agar were made and the organism was found to be still viable in all tubes. Single spore cultures were made from two of the cultures thus obtained and were compared in the same Petri dish with single spore cultures from two isolates recently obtained from diseased muskmelons. All four sets of single spore cultures appeared identical. Thus the cultural expression of the wild type had remained unchanged in dry soil for three years, and it was left to determine whether its pathogenicity had been affected. To investigate this, a greenhouse infection experiment was performed, which included four single-spore cultures, one from each of the four sets mentioned. The technique of the experiment followed that employed in the previous studies on the muskmelon wilt *Fusarium* (23). Fifty seeds (variety Hoodoo) were planted per flat and emergence in the two check flats was very high (98%). Fifteen days after the melon seeds were planted the percentage of mortality (including both pre- and post-emergence killing) caused by the two cultures from the soil tubes was 88 and 90%, respectively, and that caused by the two 1945 isolates 82 and 84%. Twenty days after planting these percentages were 100, 98 and 98, 100, respectively. It may therefore be concluded that the wild type underwent no change in cultural form and suffered no loss of virulence during three years in dry soil.

The other *Fusaria* studied here have not been investigated as thoroughly as the muskmelon *Fusarium*, but it may be stated that the wild types of the soybean, tomato, aster, navy bean, flax, cotton, and cowpea *Fusaria* were still viable in similar tubes after 19 months. *Fusarium moniliforme* was found to be still viable after 15 months, and the two soil *Fusaria* after 13 months.

To replace the normal laboratory technique of maintaining stock cultures of *Fusaria* by serial transfers on media high in nutrient, the following procedure is suggested. Inoculate tubes of moist sterilized soil with recently obtained mass isolates or single spore cultures and allow contents to become dry. Transfer once a year to a fresh tube of soil. As a safeguard the purity may be checked at each transfer. Such tubes should provide a reliable source of inoculum for studies on pathogenic behaviour and on taxonomy.

With regard to the problem of exsiccatae it does not seem unreasonable that soil cultures could serve as type specimens for herbaria. Wollenweber *et al.* (44) state that pure cultures of each of the *Fusaria* studied at the *Fusarium* Conference would be placed in the Bureau of Plant Industry, U. S. D. A. and would have substantially the status of type specimens. It is difficult to conceive of what taxonomic value such cultures could be, particularly since the "normal culture" technique of the authors probably resulted in the loss of the wild types even before the cultures were placed in the Bureau. Rather, it reaffirms the necessity of developing some convenient method for

maintaining the wild types in the pure state, if a satisfactory degree of uniformity is to be introduced into the taxonomy of the genus.

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## CULTURAL AND TAXONOMIC STUDIES ON CERTAIN FUSARIA

### II. THE TAXONOMIC PROBLEM IN *FUSARIUM* WITH PARTICULAR REFERENCE TO SECTION *ELEGANS*<sup>1</sup>

BY J. J. MILLER<sup>2</sup>

#### Abstract

The conclusions that the 'normal culture' stage described by certain specialists in *Fusarium* taxonomy represents the dominance of the original or wild type by a mutant, and that the 'sporodochia' that appear in laboratory cultures of *Fusarium* are actually patch mutants are shown to have an important bearing on the taxonomy of the genus. The indication is that the species descriptions of many *Fusaria* are based to a large extent on cultural variants of the types found in nature. It is emphasized that in order to avoid confusion, care should be taken to base species descriptions on the characters of the wild types. Further, while morphological separation of wild types may be found extremely difficult within certain groups of *Fusaria* owing to the close similarity of the components, the effort should not be abandoned because of the wide range of variation displayed by these organisms in artificial culture. Species should be merged only when morphological separation is extremely difficult or where variability in nature makes consistent diagnosis uncertain.

#### Introduction

Basic to a useful systematic study of any group of organisms is an understanding of the degree of variation that may be expected among them in consequence of environmental or genetic differences. In *Fusarium* it would appear that these two sources of variation are rather closely associated since, in addition to the fact that the appearance of the mycelium and the character of the sporulation of a given pure culture may be markedly affected by different types of cultural environment, certain cultural media favour the appearance of mutations that may soon dominate the original. In preceding publications of the writer (3, 4, 5) it was concluded that the taxonomy of the genus should be based on the types found to occur in nature, since otherwise a great deal of confusion might easily ensue. The phenomenon of 'cultural degradation' has, therefore, an important bearing on the problem of *Fusarium* taxonomy in two respects: (1) past systematic work on the genus should be critically examined from this point of view, and (2) future descriptions of species of *Fusarium* should be prepared in full cognizance of the confusion that may result through mutation in pure culture.

#### The Classification of *Fusarium*

Two major systems of *Fusarium* taxonomy are in existence; that embodied by Wollenweber and Reinking in their classic monograph *Die Fusarien* (14),

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and the subsequent revision of the genus by Snyder and Hansen (10, 11, 12) in which the 65 species listed by Wollenweber and Reinking were reduced to 10.

The former of these is based primarily on the work of Appel and Wollenweber (1), Sherbakoff (9), and Wollenweber *et al.* (15). These authors take the view that *Fusaria* when first isolated from nature may be in a state unsuited to taxonomic work, the so-called "Ankulturr", because the conidia are irregular in shape and septation. By growing them in culture a further stage, termed "Normkulturr", is obtained, which is characterized by the production of so-called "normal" conidia. As Sherbakoff (9) states: "A culture is considered to be a normal one (*Normkulturr*) when all forms typical of the fungus—and especially the most important form, the macroconidia,—are abundant, comparatively uniform in size and shape, smooth in outline, and so forth." He considers a culture to be in the state of undevelopment or "Ankulturr" when mycelial growth predominates accompanied by poor production of spores: "This state of culture may sometimes exist when a fungus is transferred from a mycelial growth, especially when it is taken directly from the host tissues." Again, Wollenweber *et al.* (15) take the view that generally the "normal condition of *Fusaria* from the standpoint of determination" is not present in nature but must be produced by growing the fungi in pure culture. Certain *Fusaria*, including *F. Scirpi*, are considered to be identifiable as they occur in nature, but "Other *Fusaria*, which appear primarily in a microconidial stage under natural conditions and produce only a few sickle-shaped spores, are more difficult to identify. For these the so-called normal growth must, at first, be produced." The methods proposed to effect this involve culturing the fungi over a period of time, species that persistently produce only microconidia often requiring special treatment such as repeated transfer of occasionally found macroconidia, which may finally lead to the abundant production of "normal macroconidia".

When these observations are viewed in the light of the present series, the conclusion will at once be apparent that these authors are actually dealing with genetic variants appearing in originally pure cultures. The statement of Wollenweber *et al.* (15) that "the type of inoculum influences the resultant growth" is clearly indicative of genetic differences between adjacent regions in the cultures studied.

The so-called 'sporodochia' appearing in cultures of *Fusarium* are also regarded as a useful source of macroconidia for taxonomic purposes, but evidence presented in the preceding paper of this series (5) pointed to the conclusion that these are actually 'patch mutants' that result from the invasion of the surrounding wild type by mutants. Indeed, the grouping of *Fusarium* with the Tuberculariaceae of the Fungi Imperfecti may be called into question on this basis, since it would obviously be unreasonable to group *Fusarium* with fungi that form true sporodochia merely because of a superficial resemblance of the patch mutants to sporodochia. However, some *Fusaria*, notably the flax wilt organism, form true sporodochia on the stems of the host, but in the muskmelon *Fusarium*, although surface sporulation on

the host may be abundant, it is commonly expressed as a fairly uniform field without definite boundaries. Possibly the explanation lies in the fact that the hard outer layers of the flax stem so hinder the passage of the hyphae of the pathogen that they are able to emerge only at weak points, the resultant localization imparting a sporodochial appearance to the tufts of sporulation. On the other hand, both organisms show the same type of growth and sporulation in culture, the conidia being borne uniformly over the mycelium with no evidence of localization. If then, we are to include these two organisms in the same genus, or even family, it would seem necessary to base the grouping on the form of growth expressed in culture, in which event the genus would properly belong in the Moniliaceae.

The importance to taxonomy within the genus of the preceding conclusions that 'normal' cultures represent the dominance of the wild type by macrospore-bearing mutants and that the 'sporodochia' are actually 'patch mutants' cannot be underestimated. Brown (2) has suggested that the transfer method of obtaining macroconidia results in the elimination of the parent by a saltant. He also points out that since the appressed forms with long conidia tend to be feebly, if at all, parasitic, it seems unreasonable to classify pathogenic *Fusaria* on this basis. If the type of macroconidium present at 'normal culture' were always the same, an objection of this nature would be mainly academic. There seems no reason, however, for expecting all mutants to produce the same type of spore, nor is this found to be the case. Not only have the macroconidia produced by different mutants of a given *Elegans Fusarium* been observed to differ in length, width, and degree of curvature, but the same mutant may produce a different type of macroconidium depending on whether it is in the pure state or mixed with the wild type in a patch mutant. The conclusion therefore seems valid that the search for macroconidia could lead to confusion in the species descriptions since there is no assurance that every investigator will obtain the same type of macroconidium, or even that the same investigator might not obtain different types in separate isolates of the same organism.

Additional evidence in support of this conclusion is provided by certain statements in the *Fusarium* literature. Wollenweber *et al.* (15) indicate that they are aware of the possibility of confusion and comment: "It should be stated also that even conidia normal in appearance often differ greatly in their size and shape according to where they are produced, whether on mycelium, over a wet surface, or in a definite sporodochium. In a typical pionnotes long conidia may be produced, and in sporodochia they may be considerably shorter". Similarly Reinking and Wollenweber (8) state: "Sometimes, spores of particular species (*Fusarium cubense*) may have slenderer and longer spores in pionnotal masses than when produced from the mycelium or in older sporodochial masses." And also: "The measurements of conidia on different media, especially when the spores were taken from different sources, such as mycelium, pionnotes, or sporodochia, are significant in that they point out the possible differences that may arise within the same

species when normal spores are produced under various conditions." Thus, even if it were conceded that the 'normal stage' should be employed in *Fusarium* taxonomy, the problem of deciding which type of macroconidium should be regarded as 'normal' would still have to be faced.

In this respect the views expressed in the literature are for the most part disappointingly vague. Sherbakoff (9) recommends growing the fungi "for a long period of time, under different cultural conditions and from different kinds of material for inocula. Then, after observation of all the stages, it will be possible to pick out easily the typical and normal one and base judgment mainly on that." Wollenweber (13) states that the normal stage can be recognized because it "has the highest constancy in average size, curvature, septation, colour, and is further characterized by longevity." Wollenweber *et al.* (15) do not appear as confident that the problem can be easily solved but advise taking an average: "it is necessary to pay particular attention to the average size of different types as grown upon different media and under different conditions." The impractical nature of this advice is obvious since the determination of a single *Fusarium* isolate may become a research problem in itself.

The attitude basic to the systematic treatment of the genus in "Die Fusarien" may be summed up in the words of Wollenweber *et al.* (15): "The ordinary procedure in determining *Fusaria* is to illustrate and measure normal spores and a few exceptions that show the variability and changes with increasing age." In view of the foregoing discussion it is permissible to question whether the descriptions given in "Die Fusarien" can be of much value in the identification of those *Fusaria* that do not produce macroconidia as isolated from nature. The so-called "Ankultur", which is characterized by abundant mycelial production and small conidia, would correspond to what is here termed the "wild type", and hence it appears that the 'normal macroconidium' technique has led to the exclusion of the wild types of many *Fusaria* from the species descriptions.

Snyder and Hansen (10, 11, 12), realizing that isolates of *Fusarium* may show a wide range of variation in culture, have proposed a simplification of the Wollenweber-Reinking classification. They found that the variation in the progeny of single spore cultures of certain Elegans *Fusaria* was so great that they could be fitted into more than one species or section of the genus, and a morphological separation of these forms was concluded to be impractical. They therefore converted the section Elegans into a single species: *Fusarium oxysporum*, containing a large number of biologic forms that may be distinguished by pathogenicity tests. Subsequently the remainder of the genus was similarly revised.

The conclusions drawn by the writer in the present and preceding studies (3, 4, 5) do not support the thesis of Snyder and Hansen in all respects. It is agreed that the cultural variants of Elegans *Fusaria* may overlap, but the possibility that the original or wild types may be morphologically separable should be given serious consideration. If this is found to be the case, the

Snyder and Hansen classification will certainly be unsatisfying to the taxonomist. In this connection it must be pointed out that the observations of the present series provide strong support for the wild type concept. The initial isolates of all 13 *Elegans* *Fusaria* studied were of the 'raised' type and between them differences were apparent that, though often small, were consistently maintained provided care was taken to prevent the originals from being displaced by mutants. With the muskmelon *Fusarium* the writer (3) has shown that not only were the cultural variants of this organism consistently less virulent than the wild type, but when such mutants were actually introduced into the field along with the wild type, after a year they were found to be negligible as a disease factor but the wild type still caused wilt. The fact that the organisms were found to be more stable in sterilized soil than on a commonly-employed laboratory medium (potato dextrose agar) is evidence for stability in nature. The Ankultur-Normkultur technique of the earlier workers is further evidence for the validity of the wild type concept. The fact that they found it necessary to transfer or otherwise manipulate the initial isolates of many *Fusaria* before a 'stage' producing 'normal macroconidia' could be obtained indicates that the organisms exist in nature as forms that may be altered by a period of growth in culture.

The crowding-out of the original types in culture by mutants is obviously a very widespread phenomenon in the genus and it may be questioned whether the different morphological types that thus appear are of any great taxonomic significance. In the taxonomy of *Drosophila*, for instance, the variants obtained in the laboratory are not incorporated into the systematics of the genus, but the various species are separated on the characteristics of the types found in nature. There seems no reason why a similar attitude should not be adopted in the case of *Fusarium*. It is possible, of course, that some members of the genus would be found to display a wide range of variability in the field, and if so, such organisms would present difficulties. It may be concluded, however, that in systematic studies of *Fusaria*, close attention should be paid to the degree of variation displayed *in nature*, the mutants that arise under the highly abnormal conditions of artificial culture providing no sound basis for the broadening of species descriptions.

### Taxonomic Characters in the Section *Elegans*

Since the wide range of variability shown by the *Elegans* *Fusaria* in culture renders it advisable that only the original types be considered in a taxonomic treatment, it is important to know whether these are sufficiently distinct to permit separation on morphological grounds. While the *Elegans* *Fusaria* studied by the writer were closely similar in certain cases, in others differences were consistently apparent, and these were investigated to determine to what extent they would permit a convenient morphological separation of the organisms concerned.

The size and shape of conidia have hitherto been regarded as highly important taxonomic characters in *Fusarium*. All the Elegans Fusaria studied here produced mainly microconidia on potato dextrose agar. But Wollenweber *et al.* (15) do not regard these spores as of much diagnostic value except in certain cases where they have definite characters such as pear-like shape or formation in chains. Since it is not permissible to employ the macroconidia that often appear abundantly in patch mutants, it follows that one should either measure and compare the size and shape of the microconidia, or search for a medium on which the wild type mycelium will produce macroconidia.

To investigate the former alternative, an experiment was performed with five Elegans Fusaria to determine to what extent these are separable by the characteristics of the microconidia produced on potato dextrose agar. The muskmelon, tomato, and navy bean Fusaria, and Soil Fusaria A and B were grown in the same Petri dishes, which were kept at 27° C. in an incubator inside which the humidity was maintained at a fairly high level by strips of blotting paper, the lower ends of which were immersed in water. From each of the wild types, 100 spores were measured at intervals of 4, 8, 12, 16, and 26 days after the plates were inoculated; the results of the experiment are summarized in Table I.

Considering first the effect of age of culture on spore size, it is noted that in all five organisms the tendency is for spore size to decrease as the cultures age. Two explanations may be advanced to account for this. The spores may actually shrink through loss of water or smaller spores may be cut off by the conidiophores as the supply of nutrient in the medium became exhausted. It is worth noting in this connection that all the conidia produced by a single conidiophore are not identical in size but show about as much variation as that existing between spores from different conidiophores. It was possible to make this observation since the spores from individual conidiophores tended to cling together in small groups. In view of the progressive decrease in average size of the spores, it would seem wise to decide upon an arbitrary age of cultures at which measurements should be made. Since on the whole the rate of decrease was greatest during the first 16 days, after which the average size tended to remain relatively constant, two weeks would perhaps be a suitable age for the taking of spore measurements.

It will be apparent from the table that the spore sizes fall into two well-defined groups. The spores of the first group, which includes the muskmelon *Fusarium* and Soil Fusarium B, are longer than those of the navy bean and tomato Fusaria and Soil Fusarium A, which comprise the second group. This difference affects the ratio of width/length, causing it to be lower in the first group. Within the groups differences do not seem as apparent. In the second group, for instance, the tomato *Fusarium* conidia seem on the average shorter than those of either the navy bean *Fusarium* or Soil Fusarium A. In the first group the spores of the muskmelon *Fusarium* seem slightly

TABLE I

COMPARISON OF THE DIMENSIONS OF THE NONSEPTATE CONIDIA PRODUCED BY FIVE *FUSARIUM* WILD TYPES ON POTATO DEXTROSE AGAR. EACH AVERAGE INVOLVES 100 CONIDIA

Organism	Age of culture, days	Maximum ( $\mu$ )	Minimum ( $\mu$ )	Average ( $\mu$ )	Ratio: Width/Length
Muskmelon <i>Fusarium</i>	4	13.3 $\times$ 3.6	5.5 $\times$ 2.4	8.68 $\times$ 3.15	0.36
	8	12.4 $\times$ 3.1	5.5 $\times$ 2.7	8.02 $\times$ 3.01	0.38
	12	13.8 $\times$ 3.6	5.8 $\times$ 2.7	8.36 $\times$ 3.09	0.37
	16	10.9 $\times$ 2.7	4.7 $\times$ 2.5	7.45 $\times$ 3.06	0.41
	26	10.5 $\times$ 3.5	4.9 $\times$ 2.7	7.49 $\times$ 2.97	0.40
Tomato <i>Fusarium</i>	4	11.3 $\times$ 3.6	4.0 $\times$ 2.9	6.45 $\times$ 3.13	0.49
	8	8.3 $\times$ 3.3	4.0 $\times$ 2.0	5.65 $\times$ 2.97	0.53
	12	7.8 $\times$ 3.5	4.4 $\times$ 2.5	5.97 $\times$ 3.19	0.54
	16	8.7 $\times$ 4.0	3.8 $\times$ 2.9	5.88 $\times$ 3.13	0.53
	26	10.2 $\times$ 3.8	4.2 $\times$ 2.5	5.70 $\times$ 3.04	0.53
Navy bean <i>Fusarium</i>	4	10.7 $\times$ 3.6	4.0 $\times$ 2.9	6.77 $\times$ 3.13	0.46
	8	9.3 $\times$ 3.6	3.6 $\times$ 2.2	6.07 $\times$ 3.09	0.51
	12	8.4 $\times$ 3.6	4.4 $\times$ 1.8	5.90 $\times$ 2.89	0.49
	16	9.5 $\times$ 3.6	4.2 $\times$ 2.2	6.18 $\times$ 2.96	0.48
	26	9.8 $\times$ 3.5	4.4 $\times$ 2.4	5.98 $\times$ 2.78	0.47
Soil <i>Fusarium A</i>	4	14.6 $\times$ 3.6	4.5 $\times$ 2.5	8.79 $\times$ 3.29	0.38
	8	11.3 $\times$ 3.6	4.0 $\times$ 2.7	6.88 $\times$ 3.36	0.49
	12	12.0 $\times$ 4.0	4.4 $\times$ 2.9	6.82 $\times$ 3.27	0.48
	16	10.4 $\times$ 4.0	4.0 $\times$ 2.5	5.94 $\times$ 3.09	0.52
	26	10.6 $\times$ 4.4	4.0 $\times$ 2.4	6.11 $\times$ 3.13	0.51
Soil <i>Fusarium B</i>	4	12.2 $\times$ 3.3	5.5 $\times$ 2.2	8.54 $\times$ 2.87	0.34
	8	13.5 $\times$ 4.0	5.8 $\times$ 2.2	7.94 $\times$ 2.76	0.35
	12	10.9 $\times$ 2.9	5.5 $\times$ 2.2	7.62 $\times$ 2.86	0.38
	16	13.1 $\times$ 4.0	5.5 $\times$ 1.8	7.99 $\times$ 2.94	0.37
	26	11.3 $\times$ 3.1	4.5 $\times$ 1.8	7.49 $\times$ 2.68	0.36

wider than those of Soil *Fusarium B*, which is reflected in the ratio of width/length, but these separations are neither as sharp nor as consistent as those existing between the two main groups. However, in the latter an additional feature of the spores may be employed to effect a separation, since the conidia of Soil *Fusarium B* were often narrower at one end. In addition, in Soil *Fusarium B* about 2% of the conidia were septate, having one, two, or even three septa. Septate spores were very rare in the other four *Fusaria* on this medium. The conclusion seems reasonable that, while in many instances a separation may be effected on the basis of spore measurements, it will often be necessary to employ further characters either of the spores or of cultures to distinguish the various members of the section *Elegans*.

Considering first, in this connection, the spores produced on potato dextrose agar, characters other than size that should be of importance are shape and abundance. The value of a characteristic shape has been indicated above with Soil *Fusarium B*. With regard to abundance, when a small amount of aerial mycelium from the tomato *Fusarium* is placed in a drop of water

on a slide and flattened with a coverslip, a marked cloudiness is noted, which proves to be caused by the enormous number of microconidia borne on the mycelium. This effect was not obtained with the muskmelon *Fusarium*. Soil *Fusarium B* resembled the muskmelon *Fusarium*, while Soil *Fusarium A* and the navy bean *Fusarium* sporulated very abundantly though they did not seem to equal the tomato *Fusarium* in this respect.

It was observed that when test-tubes of moist sterilized soil were inoculated with the various *Elegans Fusaria* studied here, a small amount of loose aerial mycelium appeared above the surface of the soil. On this mycelium spores were borne that varied from small non-septate to large several-septate conidia. Since the macroconidia here are produced by the wild type, there would be no objection to employing them taxonomically. Some differences were apparent, the macroconidia of the muskmelon *Fusarium* being longer and less pointed at the ends than those of the navy bean *Fusarium*, but no experiment was performed to investigate these differences more fully. The indication is, however, that features displayed by the wild types on media other than potato dextrose agar should be of taxonomic value.

The general appearance in culture, including the appearance of the aerial mycelium and the pigmentation of the immersed mycelium, differs in many cases. The former, however, is always difficult to describe in words and the latter was found to be very unreliable, varying even with different lots of the same type of medium.

The 'sclerotia' that often appear in cultures of *Fusaria* have already been discussed (5). Not all the *Fusaria* studied here seemed to produce these but in cultures of the muskmelon, pea, and soybean *Fusaria*, and Soil *Fusarium A* they were abundant, as many as 200 appearing in a single Petri dish culture. It must be realized that since these appeared to be of mutant origin, indicating genetic instability in the organisms that produce them, it may actually be the latter character that is being compared, but there seems no reason why the mutation frequency of a given organism on a given medium should not be treated as a taxonomic character. Likewise, the frequency and general appearance of patch mutants should not be overlooked in a taxonomic study. Patch mutants were very abundant and conspicuous (on potato dextrose agar) in the muskmelon, pea, cabbage, and flax *Fusaria*.

The taxonomic value of the phenomenon termed 'cultural interaction' has been discussed in a preceding publication (5), where the conclusion was reached that it is sufficiently specific to be an aid both in diagnosing *Fusaria* and in providing a check on diagnoses arrived at by other criteria, particularly in the case of saprophytes.

The evidence thus indicates that characters of taxonomic value, both morphological and physiological, are by no means infrequent in the wild types of *Elegans Fusaria*. It is hoped that the present studies will lead to the establishment of a morphological classification that will avoid confusion by adhering strictly to the wild types and that may be correlated with pathogenicity trials in the case of parasites.

An indication of the way in which this may be approached is provided by the key that follows. It will be noted that a final separation is not effected in certain cases, but perhaps the characters expressed on other media would help. Spore measurements may also prove useful here. With the cotton and cowpea *Fusaria* a very sharp separation is provided by cultural interaction as was shown in the preceding paper of this series (5). 'Type' organisms would be useful in interpreting keys of this nature. The muskmelon and tomato *Fusaria* could be employed in this respect, the former exemplifying sparse sporulation and the abundant and conspicuous production of patch mutants and sclerotia, and the latter exemplifying profuse sporulation and infrequent production of patch mutants and sclerotia. It seems apparent that much careful taxonomic work is required before an accurate classification of this section, and perhaps of others as well, may be drawn up.

### Morphological Key to 13 *Elegans* *Fusaria* Based on the Features Expressed on Potato Dextrose Agar

- A. Sporulation on aerial mycelium sparse
  - B. Sclerotia abundant.....Muskmelon *Fusarium*
  - BB. Sclerotia very few or absent
    - C. Patch mutants abundant and conspicuous
      - D. Patch mutants appear as white, cottony areas in aerial mycelium  
Flax *Fusarium*
      - DD. Patch mutants appear white in aerial mycelium but are dense and extend into the immersed mycelium.....Cabbage *Fusarium*
    - CC. Patch mutants infrequent.....Soil *Fusarium* B
- AA. Sporulation on aerial mycelium profuse
  - B. Sclerotia abundant
    - C. Plectenchymatic patch mutants present
      - D. Sclerotia irregularly distributed through medium....Soil *Fusarium* A
      - DD. Sclerotia regularly distributed in the sense that they are either at the surface of the medium or are deeply sunken in the medium, adjacent to glass of Petri dish.....Soybean *Fusarium*
    - CC. Plectenchymatic patch mutants very few or absent.....Pea *Fusarium*
  - BB. Sclerotia very few or absent
    - C. Plectenchymatic patch mutants present.....Aster *Fusarium*  
Navy bean *Fusarium*  
Gladiolus *Fusarium*
    - CC. Plectenchymatic patch mutants very few or absent
      - D. Mottled appearance developing after two or three weeks in the immersed mycelium.....Cotton *Fusarium*  
Cowpea *Fusarium*
      - DD. Mottled appearance not developing in the immersed mycelium  
Tomato *Fusarium*

### Discussion

It seems apparent that a great deal of confusion has arisen in the taxonomy of the genus *Fusarium* as a consequence of mutation in pure culture. Snyder and Hansen (12) describe many instances of apparent transmutation of species through variation. Padwick (6) found that duplicate tubes of various



*Fusarium* cultures varied greatly in the ratios of continuous to septate spores. In fact, his very thorough taxonomic work on part of the section *Elegans* led him to state: "We are forced to the conclusion that the cultures of *Orthocera-Fusaria* maintained at Baarn cannot, with one or two exceptions, be recognized from the descriptions given by Wollenweber and Reinking." The work of the present writer (3, 4, 5) has confirmed the observations of Snyder and Hansen and of Padwick in that variants widely different from the original were obtained in culture, and a number of generalizations seem deducible from this work that are of significance with regard to the taxonomy of the genus. (1). In most cases the initial isolates of a given *Fusarium* are identical, or closely similar, in culture. (2). When grown in pure culture on a laboratory medium such as potato dextrose agar, the original or wild type is soon displaced by mutants that are more successful than the 'parent' in this highly abnormal environment. (3). The process seems unidirectional, no instance of reversion having been noted after a given mutant was purified by the single-spore method. It would thus seem advisable to base the taxonomy of the genus on fresh isolates of the organisms, unless particular care is taken to maintain these in the pure state. Otherwise there appears little hope that a usable morphological treatment of *Fusarium* will be developed.

Snyder and Hansen (12) in discussing the Wollenweber and Reinking classification (14) make the statement: "Another serious breakdown in the system occurs when fungi which originally may have been specifically identified are re-examined and re-identified after a period in culture, for then it may be found that the first and second identifications do not agree, perhaps not even in respect to section characters". This is an agreement with the foregoing generalizations. However, the conclusion drawn by Snyder and Hansen, that this situation necessitates the merging of species and abandonment in many instances of all hope of morphological separation does not necessarily follow, since it must first be proved that the process occurs in nature as well as in artificial culture. Padwick (7) has criticized the action of Snyder and Hansen in converting the section *Elegans* into a single species. He states that they have reduced the possibility of making errors by the simple procedure of being vague. Additional reasons given for not accepting this revision are (1) that Snyder and Hansen assume Wollenweber and his associates to be wrong in their grouping of isolates into species, yet right in their grouping of species into sections, and (2) they tend to lose objectivity by concentrating on the potentialities of the species in test-tubes rather than on its objective existence in nature. To clarify the taxonomic situation in *Fusarium*, Padwick proposes the concept of "focal point species", which involves the study of *Fusaria* in their natural habitat. This appears similar to the 'wild type' concept of the present writer, and some such revision of the basis for taxonomy in the genus would seem advisable.

It is conceivable that the objections raised in the present series with regard to both prevailing systems of *Fusarium* taxonomy may lead to a reconciliation between the two. Possibly a system based on morphology would be to a

large extent successful and useful, provided only the wild types are included. But where differences are very slight, or else are confused by the presence in nature of a number of cultural strains, the merging of species as proposed by Snyder and Hansen would be desirable.

### Acknowledgments

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## THE MICROFLORA OF STORED WHEAT<sup>1</sup>

BY NORMAN JAMES<sup>2</sup>, JOYCE WILSON<sup>3</sup>, AND EGON STARK<sup>4</sup>

### Abstract

Wheat passing through the Winnipeg market in 1943 and 1945 harboured a large number of micro-organisms. They were not found on all kernels, but were present on all 10-gm. samples. The number was smallest on No. 1 Manitoba Northern red spring wheat and progressively larger on each successive lower grade.

The organisms consisted chiefly of two species of bacteria, which persisted on the grain even after repeated vigorous washings. One was identified as *Bacterium herbicola aureum* Duggeli. The other undoubtedly is a representative of the genus *Pseudomonas*. It is suggested that these species should be considered epiphytic on wheat.

### Introduction

Although grain would be expected to harbour bacteria, yeasts, and fungi that might affect its keeping quality, little information on the saprophytic flora of stored wheat is available. Duggeli (1904 (5, 6) ) reported that healthy grain kernels harboured on their surfaces very large numbers of bacteria belonging mainly to one species. Morgenthaler (1918 (12) ) found bacteria ranging in numbers from 80,000 to 25,000,000 per gm. on normal or healthy wheat; and fungi ranging from 4000 to 7200 per gm. He claimed that healthy kernels were free from fungi and that injury to the seed coat of some kernels was responsible for the small fungal population on the weighed sample. Mack (1936 (10) ) counted the bacteria on sprouted and unsprouted wheat. The number of bacteria on 20 kernels of unsprouted wheat averaged 60,000 per kernel; and on 20 kernels of sprouted wheat 1,300,000 per kernel.

Kent-Jones and Amos (1930 (8) ) made estimates of bacteria on 21 samples of wheat passing through the normal channels of trade in Great Britain. Estimates from nutrient agar plates, incubated at 37° C. for 48 hr., ranged from 8000 to 8,000,000 per gm. of wheat.

Gustafson and Parfitt (1933 (7) ) reported counts ranging from 46,000 to 3,260,000 per gm. on 19 samples of wheat investigated.

The significance of the flora on wheat is of interest at this time because of the somewhat general belief that micro-organisms cause the heating of grain under certain conditions of storage (9, 11, 14) and because of the large surplus of wheat stored in elevators during the past decade.

This report deals with isolations of bacteria, yeasts, and filamentous fungi from different grades of stored wheat by the technique of the bacteriologist interested in the general, rather than the pathogenic, flora of grain.

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## Samples and Methods

Samples of four grades of Manitoba Northern red spring wheat and of No. 5 and No. 6 wheat were supplied through the courtesy of the Chemist-in-Charge, Grain Research Laboratory, Board of Grain Commissioners for Canada. They represented composites of all wheat of these grades passing through the regular channels of trade in Winnipeg in certain two week periods in the summer of 1943. Each sample consisted of about 150 gm. Tests were made on six replicate 10 gm. portions of each sample. Each portion was transferred aseptically to a 6 oz. screw-top bottle containing 100 ml. of sterile water and about 10 gm. sterile gravel and was shaken for 30 min. on a to-and-fro shaking machine. Appropriate dilutions were made; and duplicate plates were prepared in a special transfer room previously sprayed with a disinfecting solution. Standard nutrient agar was used as a substrate for bacteria; and Czapek's agar, plus 0.5 ml. of 10% lactic acid per 100 ml. of medium, for yeasts and fungi. Plates were incubated at 26° C. for six days. Counts were recorded as numbers of bacteria, yeasts, and fungi in the washings from 1 gm. of wheat. The experiment was carried out four times in 1943 and repeated four times in 1945.

### Numbers of Organisms in First Washings from Wheat

The bacterial populations in the washings from the samples studied in 1943 were found to be extremely high, ranging from 280,000 to 164,000,000 per gm. of wheat; and the increase in numbers from Grade 1 to Grade 6 was strikingly regular in each replication of the experiment. Yeasts, likewise, were present in large numbers—6200 to 64,000; but not all replications showed the same trend in numbers from Grade 1 to Grade 6. On the contrary, only relatively small numbers of fungi—420 to 1870—were found in the washings; and the difference between grades appeared to show little consistency.

The trials carried out in 1945 showed essentially the same trends.

The results of the four replications of the experiment in 1943, expressed as averages of logarithms of counts, and of the four replications in 1945 are shown graphically in Fig. 1.

Since the percentage of frosted, immature, and weathered kernels is considered in establishing the grade of wheat, it appeared advisable to determine to what extent the populations on individual kernels of these types might account for the population differences in the different grades referred to in the previous experiment. From a composite sample of No. 4 Manitoba Northern red spring wheat 30 kernels of each of the following types were selected—vitreous, weathered, frosted, and immature.\* Each kernel was tested by the procedure outlined under Samples and Methods, with one exception. Ten ml. of sterile water was used, instead of 100 ml., in the initial dilution.

\* *Dr. P. J. Olson and Mr. A. J. Lejeune of the Department of Plant Science made the selection.*

In these trials, too, bacterial counts showed the widest range. On the average, vitreous kernels harboured the smallest numbers, with weathered, immature, and frosted kernels sustaining progressively increasing numbers of

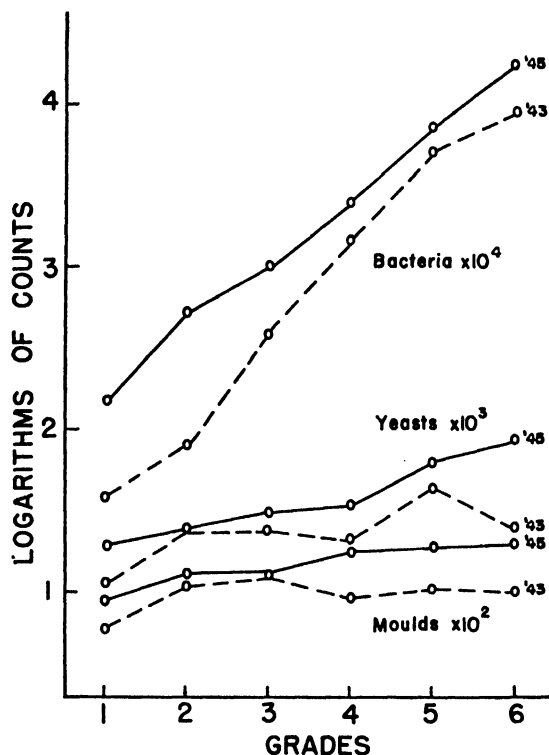


FIG. 1. Relation between grades of wheat and numbers of micro-organisms in first washings; averages of 24 determinations.

bacteria. The results on the four types, expressed as logarithms of averages from 30 kernels in each case, are shown graphically in Fig. 2. Some kernels of each type gave counts that did not conform with the general trend illustrated in Fig. 2. This is brought out in Fig. 3a, which shows the distribution of the kernels in classes based on logarithms of numbers on separate kernels. Plate counts of less than 100,000 bacteria per kernel were produced by 28 vitreous kernels whereas only 27 weathered, 19 immature, and 18 frosted kernels fell in classes under 100,000.

The yeast population on the same kernels failed to show a consistent relation to type of kernel, even though the trend for yeasts in Fig. 2 appears to indicate it. One immature kernel harboured a larger number of yeasts than the total on the other 29, which accounts for the high point for immature kernels in the figure. The class distribution of kernels based on yeast counts, shown in Fig. 3b, makes it apparent that the yeast population was highly variable. The fungal population on these kernels was small and failed to indicate a consistent difference between types of kernel, as is shown in Figs. 2 and 3c.

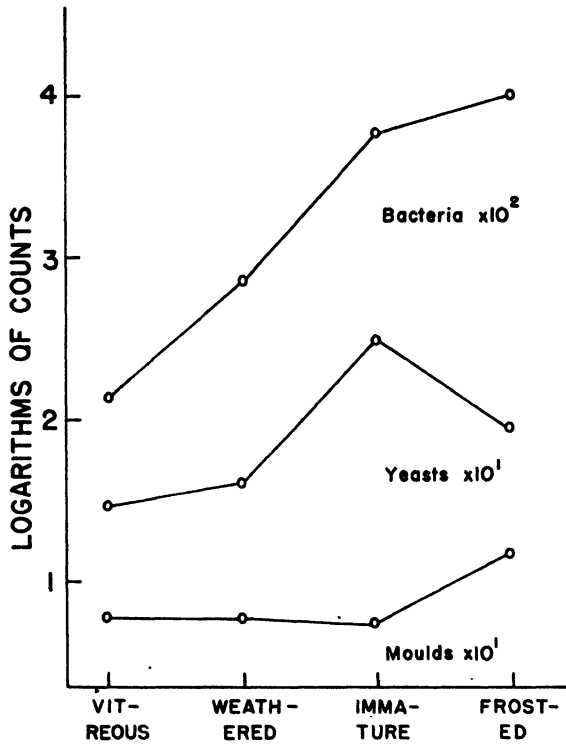


FIG. 2. Relation between types of wheat kernel and numbers of micro-organisms; averages of 30 kernels.

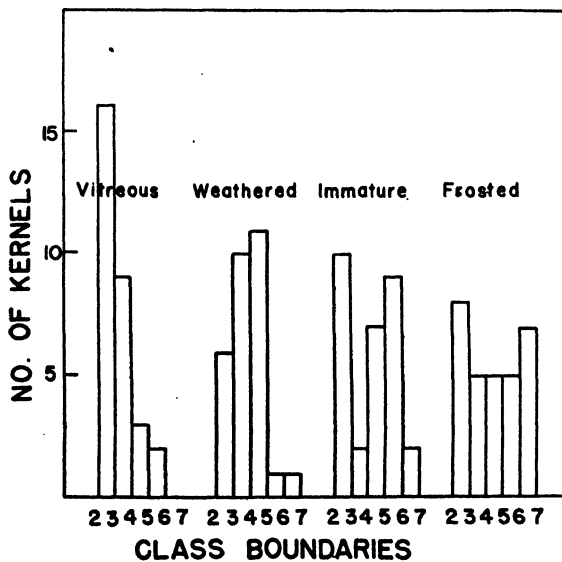


FIG. 3a. Distribution of different types of wheat kernel based on logarithms of counts of bacteria.

### Numbers of Organisms in Twelfth Washings from Wheat

This part of the study was carried out to determine the numbers of organisms on wheat after repeated washings in sterile water. Each of six 10 gm. portions from samples of each of the six grades of wheat was exposed to 12 consecutive washings in sterile water and gravel as follows: five washings for 30 min. on

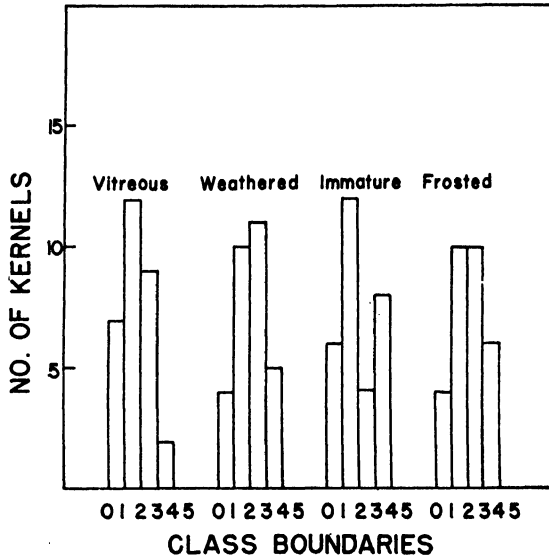


FIG. 3b. Distribution of different types of wheat kernel based on logarithms of counts of yeasts.

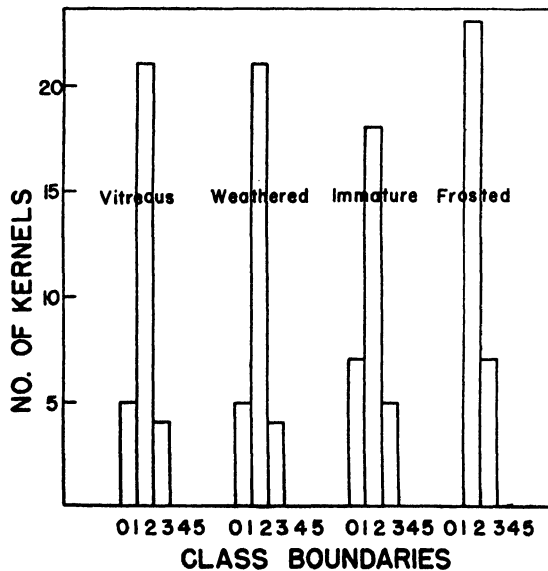


FIG. 3c. Distribution of different types of wheat kernel based on logarithms of counts of fungi.

the mechanical shaker, six rinsings, and a final washing for a 30 min. period. Appropriate dilutions for plating were made from the final wash water. The experiment was carried out twice.

The numbers of bacteria in the final wash waters were found to be high—about 20% of those found in the first washings from the samples used in the first experiment. Again, the increase from No. 1 to No. 6 grade was regular. Yeasts and fungi also were present in the final wash waters, but in relatively small numbers. The results of the two replications of this experiment are shown in Fig. 4. They present essentially the same picture as is shown for the first washings from wheat in Fig. 1.

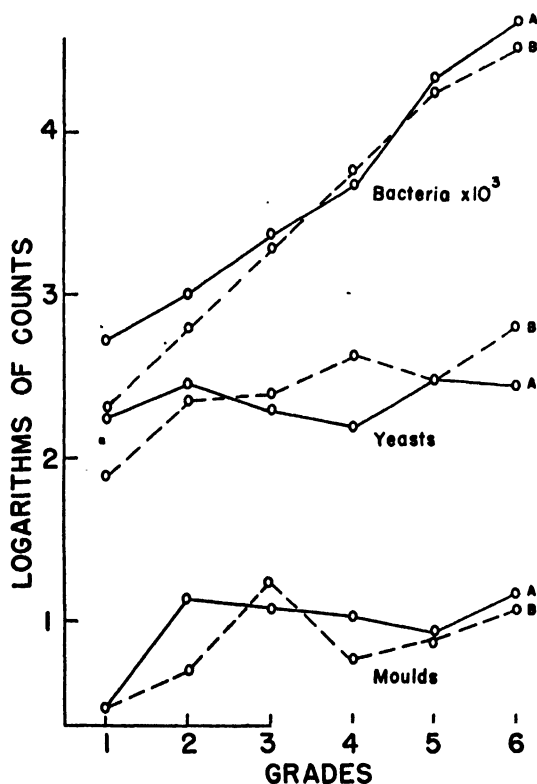


FIG. 4. Relation between grades of wheat and numbers of micro-organisms in twelfth washings; averages of six determinations.

In order to obtain direct evidence on the proportion of organisms remaining on wheat after vigorous washings, counts were made on the first and twelfth washings from a sample of No. 1 Manitoba Northern and a sample of No. 4 Manitoba Northern. The experiment was replicated three times and the averages used to indicate the proportion of the various groups of organisms in the twelfth washings. In the No. 1 grade sample, the counts in the twelfth washings were, for bacteria, 21%; for yeasts, 4%; and for fungi, 33% of the counts in the first washings; and in the No. 4 grade sample they were 37%,



3%, and 19%, respectively. It should be noted, however, that the fungal populations in the first washings were small—1400 per gm. in the No. 1 grade sample and 2900 in the No. 4 grade sample.

### Numbers of Organisms on Crushed Wheat After 12 Washings

If such large numbers of bacteria and yeasts are to be found in all washings from wheat it is evident that there must be large numbers of organisms that cannot be removed by washing. In order to get information on this point four portions of a sample of No. 1 Manitoba Northern wheat were exposed to 12 washings as outlined previously. Then after draining off the twelfth water each sample was ground with a pestle and mortar, under aseptic conditions, and raised to appropriate dilutions for plating. The results are presented in Table I.

TABLE I

NUMBERS OF ORGANISMS IN WASHINGS FROM WHEAT AND IN THE CRUSHED RESIDUE (PER GRAM)

Replicate portions	Bacteria $\times 10^3$			Yeasts $\times 10^4$			Fungi $\times 10^4$		
	1st	12th	Residue	1st	12th	Residue	1st	12th	Residue
1	108	21	630	760	10	220	15	15	60
2	102	98	120	1020	15	230	32	42	10
3	43	1.7	49	430	3	370	13	1.5	1.5
4	104	7.7	144	455	12	273	16	1.5	50

### Types of Bacteria in Washings from Wheat

Identification of all bacteria on wheat presents a problem that involves a great deal of study. Probably the best that can be hoped for is to confine the undertaking to certain types that predominate. A careful examination of colony types on plates repeatedly revealed the fact that the majority of the colonies fell in either of two groups. This was most evident on plates from the twelfth washings from wheat. Accordingly, an attempt was made to get an estimate of the relative prevalence of these two types and to identify them. For the first test all the 99 colonies developing on two plates from a one-millionth dilution of the twelfth washing from a sample of No. 6 wheat were picked into nutrient broth. After incubation each culture was examined microscopically for evidence of purity and picked to an agar slant medium for later studies. On the basis of this preliminary study, involving growth characteristics on nutrient broth and on nutrient agar, shape, size, motility, and Gram's staining reaction, 30% of the isolations proved to be of one type and 45% to be of another type, different in several respects from the former. These were designated Types *A* and *B* and will be referred to hereafter by these letters. A second test, replicated three times on a sample of No. 1 Manitoba Northern red spring wheat, gave an

average incidence of 34% for Type *A* and of 30% for Type *B*; and a third test on No. 4 Manitoba Northern gave average incidences of 37% and 40%, respectively. The remaining 23 to 36% of the colonies developing on the plates used represented what were accepted to be different types, or in some cases mixed cultures. They were not considered further.

A representative culture of each of Type *A* and Type *B* was selected, replated through two successive platings and isolations to ensure purity and investigated in detail by recognized pure culture study methods.

Type *A* undoubtedly represents a species reported by Burri (1903 (4)) to be an epiphyte normally found on the leaves of a wide variety of plants. Duggeli (1904 (5)) found the same species on a variety of seeds, including common and durum wheats. He claimed that it represented from 97 to 100% of the flora on five samples of wheat investigated. In a later report (1904 (6)) Duggeli described the species in detail and named it *Bacterium herbicola aureum*. He stressed the formation of characteristic zooglea, which could be observed as sausage-like clumps under low magnification, a phenomenon that was apparent in our isolations only after careful examination at high magnification. Mack (1936 (10)) studied several strains of the species in detail. She claimed that it should be called *Flavobacterium herbicola*, and not *Pseudomonas trifolii* as listed in the fifth edition of Bergey's manual (1939 (1)).

Type *B*, which appeared about as often as Type *A* in our isolations, evidently was not observed by Burri or Duggeli. It could be distinguished readily from the latter type by the lack of yellow pigment on surface colonies and its strong fluorescence in sucrose broth when exposed in ultra-violet light. Duggeli claimed that *Bacterium fluorescens* (*Pseudomonas fluorescens*) represented the second most prevalent species on some seeds and seedlings, but in relatively small numbers. Undoubtedly, Type *B* is a representative of the genus *Pseudomonas* that does not belong to any of the species listed in Bergey's manual. It differs from *Pseudomonas fluorescens* in its strong tendency to slime formation, its failure to develop colour on nutrient agar or in nutrient gelatine or nutrient broth, and its failure to reduce nitrates or to produce acid from dextrose.

### Types of Fungi in Washings from Wheat

A preliminary study of the types of fungi developing on Czapek's agar from the first washings from the samples investigated in 1943 was carried out.\* This involved selection of typical colonies from a large number of plates, sub-culturing on Czapek's agar slants, and identification studies, without attempting to obtain detailed information on the distribution of the types isolated. Species of *Penicillium* predominated, with *P. chrysogenum*, *P. frequentans* and *P. terrestre* occurring the most frequently. Species of *Aspergillus*, *Alternaria*, *Fusarium*, and *Cladosporium*, likewise, represented a large

\* This part of the study was carried out by Miss Wilson under the guidance of Dr. H. J. Brodie of the Department of Botany.

proportion of the cultures developing on the plates. The following types were identified: *Acrostalagmus cinnabarinus* Corda; *Alternaria tenuis* auct. sensu Wiltshire; *Aspergillus candidus* Link; *A. flavus* Link; *A. fumigatus* Fres.; *A. glaucus* Link; *A. niger* van Tiegh.; *A. Oryzae* (Ahlburg) Cohn; *A. versicolor* (Vuill.) Tirab.; *Botrytis cinerea* Pers.; *Cephalosporium curtipes* Sacc.; *Cephalosporium* spp.; *Cephalothecium roseum* Corda; *Cladosporium herbarum* (Pers.) Link; *Fusarium culmorum* (W. G. Smith) Sacc.; *F. Poae* (Peck) Wollenw.; *F. Scirpi* var. *acuminatum* (Ell. and Ev.) Wollenw.; *F. semitectum* Berk. and Rav. var. *majus* Wr.; *Helminthosporium sativum* Pam., King, and Bakke; *Hormodendron pallidum* Oudem.; *H. viride* (Fres.) Sacc.; *Monilia* spp.; *Mucor circinelloides* van Tiegh.; *M. racemosus* Fres.; *Paecilomyces varioti* Bain.; *Penicillium chrysogenum* Thom; *P. flavi-dorsum* Biourge; *P. frequentans* Westl.; *P. purpurogenum* Stoll; *P. rugulosum* Thom; *P. spinulosum* Thom; *P. terrestre* Jensen; *Phoma* spp.; *Rhizopus* spp.; *Scopulariopsis brevicaulis* (Sacc.) Bain.; *Scopulariopsis* sp.; *Septoria nodorum* Berk.; *Torula* spp.; *Trichoderma lignorum* (Tode) Harz.

All except five of the species listed above have been isolated from Manitoba soils (2, 3). The additional species obtained from wheat follow: *Cephalothecium roseum*; *Septoria nodorum*; *Fusarium semitectum*; *Aspergillus Oryzae*, and the *A. glaucus* group.

Seven of the species isolated in this study are listed in Orton's bibliography (1931 (13)) on seed-borne parasites. These follow: *Alternaria tenuis*; *Aspergillus niger*; *Cephalothecium roseum*; *Cladosporium herbarum*; *Fusarium culmorum*; *Helminthosporium sativum*; and *Septoria nodorum*.

### Discussion

The finding of such large numbers of certain species of bacteria on wheat grains points to the existence of a flora that may be termed epiphytic. As suggested earlier by Burri (4), with respect to the flora on the leaves of plants, these bacteria cannot be considered mere chance contaminants that reach the grain through air currents, insects, or other means, but must represent primarily types that develop on it. Evidently this flora is not present on every kernel, but is harboured by a sufficiently large number of them to account for the enormous population on all 10-gm. samples studied. Likewise, it is apparent that their presence cannot be attributed to any known defect in the kernel. They should be considered commensals developing on the seed-coat or in its intercellular spaces. They must be able to proliferate under the conditions of grain storage or, at least, withstand such conditions for long periods of time. What their role is in the production of the characteristic properties of cereal products or in the development of certain defects in them is not known.

Since yeasts are distributed widely in nature, particularly on fermentable materials, their presence on wheat grains might have been anticipated. The evidence of an epiphytic yeast flora on wheat is convincing since particles of soil or dust on the kernel would not be expected to harbour many yeasts. In

fact, only rarely have yeasts been found developing on Czapek's medium when soil is plated at the dilution used in this study (2, 3). However, since this epiphytic yeast flora represents relatively small numbers, which fluctuate erratically from sample to sample, it is probable that it has little practical importance.

The fungal population, on the contrary, scarcely can be considered epiphytic. It is small at the moisture level of stored wheat, which may be accepted as indicating that this population is not the result of proliferation. However, it is persistent, which probably means that spores are held mechanically and protected in the crease of the kernel or in crevices of the injured seed coat in some cases. In general, it represents species that occur commonly in soil and dust in large numbers.

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## L'ÉRABLIÈRE LAURENTIENNE

### II. LES SUCCESSIONS ET LEURS INDICATEURS<sup>1</sup>\*

PAR PIERRE DANSEREAU<sup>2</sup>

#### Sommaire

Un schéma est présenté pour définir les lignes principales de la succession dans l'aire de la forêt décidue de la Vallée du Saint-Laurent. Le climax est l'*Aceretum saccharophori laurentianum*, une forêt décidue dominée par l'*Acer saccharophorum*. Ce point final de l'évolution de la végétation régionale est atteint par cinq voies principales.

Au cours de la succession, l'amélioration graduelle du site se fait par des associations pionnières et des sous-climax. Ces derniers sont décrits sommairement quant à leurs dominantes floristiques et à leur rôle phytosociologique.

Or, les diverses successions comportent souvent un stade subterminal très proche du climax, avec lequel il se confond dans la plupart des caractéristiques essentielles, mais où l'évolution ultérieure est inhibée par une cause topographique ou microclimatique et où persistent des éléments typiques de la sère dont ils dérivent. Ces quasiclimax et le climax lui-même sont décrits assez en détail ainsi que les unions qui en caractérisent la structure.

La prisère ayant été ainsi définie, quelques éléments de la subsère sont à leur tour analysés: les érablières où s'exerce ou s'exerce l'intervention humaine. Ces disclimax sont surtout de deux types selon que la dégradation dépend du bûchage ou du pâturage. Des conditions inconnues dans la prisère sont alors réalisées qui permettent à des équilibres nouveaux de se constituer selon la capacité d'adaptation des espèces. Ces associations, ces faciès, ces unions ou ces biotopes ne doivent leur durée, évidemment, qu'à la répétition plus ou moins régulière de l'intervention.

La présente série de mémoires sur l'érablière laurentienne a pour base une analyse statistique de quadrats se rapportant à l'*Aceretum saccharophori* ou érablière nord-américaine. Les données recueillies sur le terrain devront être passées au crible de considérations diverses avant que le tableau phytosociologique puisse être publié, et réduit à une formule floristique selon les méthodes de l'école de Zürich et Montpellier (Braun-Blanquet, 1932 (3) et 1933-39 (4)).

1° Des considérations floristico-écologiques ont permis de donner une valeur d'indice à chaque espèce (Dansereau, 1943 (14)). Celle-ci, exprimée par un chiffre et multipliée par l'abondance, conduira à un estimé quantitatif du degré d'évolution de chaque station (voir Dansereau, 1945 (18) et 1946 (19)).

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\* En général la nomenclature suivie est celle de la Flore Laurentienne du F. Marie-Victorin (42). L'auteur n'a pas tenté d'adopter dans tous les cas les changements proposés depuis et justifiés par les règles internationales de la nomenclature.

2° L'histoire de l'évolution de la végétation à l'échelle régionale doit être esquissée dans ses grandes lignes, si l'on espère discerner l'orientation dynamique des principaux éléments caractéristiques. C'est l'objet du présent mémoire.

3° Les limites géographiques et par conséquent bioclimatiques de certaines espèces-clés permettront une intégration plus sûre des complexes phytosociologiques régionaux dans les biomes (Clements and Shelford, 1939)\*.

4° Diverses associations ayant été reconnues à l'aide de pareils critères, il sera possible de considérer la structure intime de chacune à l'aide de données quantitatives: couverture, stratification, abondance, fréquence, sociabilité, vitalité, fidélité, etc. (Braun-Blanquet, 1932 (3)).

5° La variation saisonnière ou annuée (Weaver and Clements, 1938 (53)) sera ensuite analysée en détail et ses aspects seront décrits quantitativement dans les divers faciès régionaux.

6° Le comportement et la représentation de certaines formes biologiques (v.g. hémicryptophytes et géophytes) seront analysés de façon à faire ressortir les modalités particulières de l'adaptation à des biocénoses déjà définies.

7° Des populations végétales et animales seront testées pour la relation géographique et écologique de leurs variations, par exemple les érables à sucre (Dansereau et Lafond, 1941 (21), Dansereau et Desmarais, 1946 (20), les violettes jaunes (Dansereau, 1945 (17)). Ceci est une application de la méthode des collections massives de Fassett (1941 (25)) mais avec un coefficient synécologique.

### La succession dans l'aire de la forêt décidue laurentienne

Le rôle écologique joué par chaque espèce est fonction de ses exigences fondamentales, de sa réaction particulière à la lumière, à la chaleur, à l'eau et au sol et plus encore aux diverses conjonctions de ces facteurs et à leur répartition dans le temps et l'espace. Ainsi, le potentiel héréditaire plus ou moins riche de chacune trouvera à s'exprimer plus ou moins complètement dans divers milieux: tantôt les virtualités atteindront leur plafond et tantôt les inhibitions seront très sévères.

Il importe de nous rappeler qu'une espèce a d'abord des *exigences* qui demandent à être satisfaites; qu'elle manifeste ensuite des *tolérances* qui lui permettent de résister à des facteurs adverses; et enfin qu'elle se montre *capable d'utiliser les ressources du milieu* (air, lumière, eau, éléments nutritifs, etc.) à des degrés divers. Ces trois niveaux d'intégrations dans le milieu permettront à chacune de s'insérer à un stade plus ou moins avancé dans l'évolution de la végétation de la ou des régions où elle croît. Les exigences d'une espèce la limitent donc à un minimum pour chacune des conjonctions des facteurs physiques. Sa tolérance lui permet de résister à un maximum d'éléments ou facteurs adverses. Mais c'est sa vitalité et son agressivité qui déterminent la niche et souvent le biotope qu'elle occupera dans chaque habitat.

\* Clements, F. E. and Shelford, V. E. *Bio-ecology*. Wiley & Sons, New York. 1939.

Par conséquent, la place habituelle d'une espèce dans la succession est un excellent indice de sa valeur écologique, et considérée en rapport avec ce que nous connaissons de ses exigences et de sa tolérance, elle nous permettra d'interpréter le dynamisme de chaque faciès.

Dans un premier mémoire (Dansereau, 1943 (14)), la valeur d'indice de 346 espèces trouvées dans des érablières laurentiennes avait été établie en fonction de leurs réactions à la lumière et à l'eau et de leur relative fidélité à la forêt décidue. L'extension géographique et les principaux traits climatiques de l'aire dont il est question ici ont été délimités dans ce premier mémoire également (Dansereau, 1943 (14), Fig. 6). Un grand nombre de relevés ont été faits depuis lors, y compris des études sur les associations autres que le climax et dans des régions en dehors de la Vallée moyenne du Saint-Laurent. Il sera donc utile d'esquisser l'ordre de la succession et d'en définir les étapes les plus importantes. On verra ensuite dans laquelle ou lesquelles de ces communautés chaque espèce atteint son optimum, où elle apparaît (seuil inférieur) et où elle disparaît (seuil supérieur).

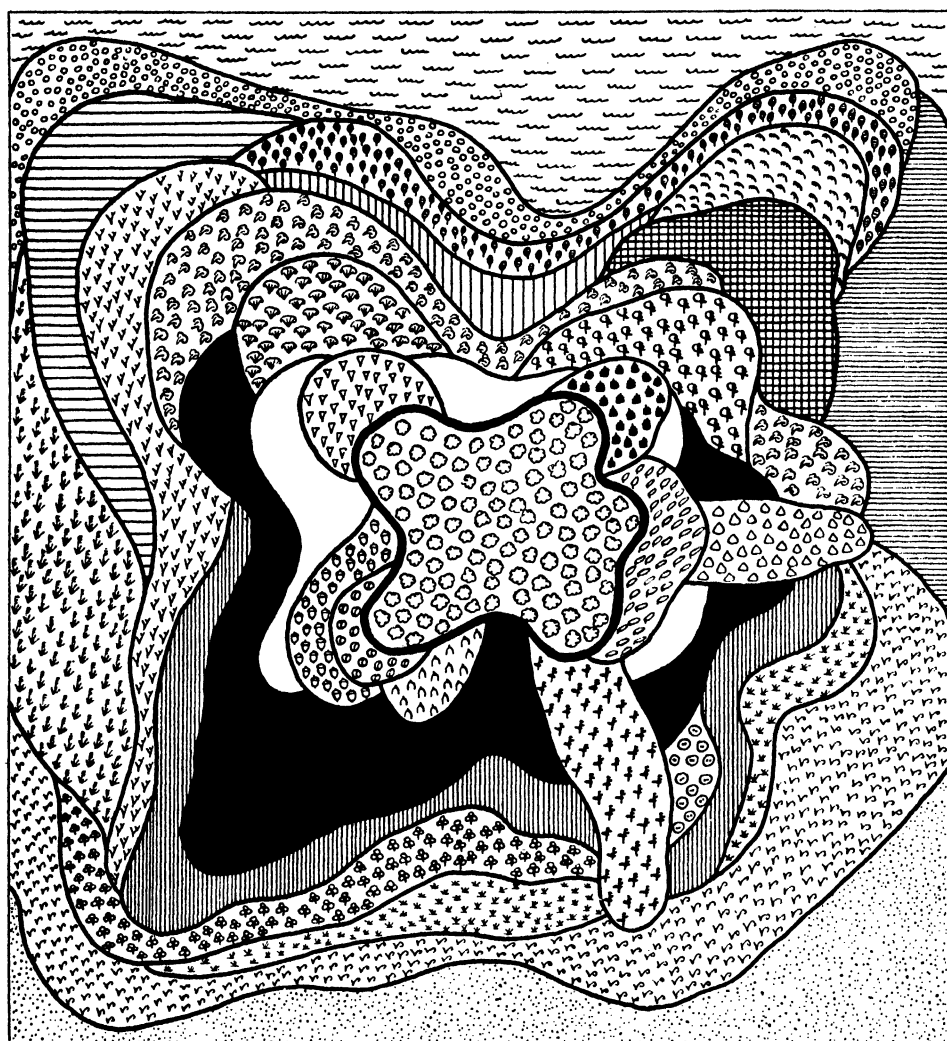
On reconnaîtra donc, soit dans la xérosère soit dans l'hydrosère, les associations pionnières, les sous-climax, les proclimax et les disclimax tous ordonnés en fonction du climax. Pour le moment, les tableaux d'association complets ne seront pas présentés. Les principaux stades seront définis par les facteurs physiques qui les conditionnent et caractérisés par les espèces les plus importantes de chaque synusie. Surtout, les relations dynamiques qui les relient seront indiquées. Il me semble que pareille considération de la valeur d'indice et de l'ordre des successions est préalable à une définition formelle et quantitative des associations.

La Fig. 1 représente une carte généralisée où le climax occupe le centre. L'hydrosère a son point de départ au sommet de la carte, et la xérosère au bas. La proximité du centre indique, pour une zone quelconque, le degré d'évolution écologique du stade qu'elle représente. Il est bien connu qu'au cours de toute succession, il peut se produire des télescopages, qu'un stade particulier peut être tellement bref qu'à toutes fins pratiques il sera escamoté, ses espèces caractéristiques étant ordinairement dispersées à l'état de reliques dans l'association suivante ou de pionnières dans la précédente. Cela engendre des contacts nouveaux entre deux groupements phytosociologiques normalement séparés l'un de l'autre. Ainsi, une prairie de danthonies peut être envahie plus ou moins immédiatement par les bouleaux sans passer par les intermédiaires de graminées, de verges d'or et de spirées. Alors, la verge d'or serait pionnière dans le *Danthonietum* et relique dans le *Betuletum*.

Il n'a donc pas été possible de représenter dans cette figure toutes les relations possibles des associations entre elles au cours de leur marche dans les diverses avenues suivies par les successions. D'autre part, je crois qu'il ne manque aucune étape importante ou usuelle.

Le phénomène de la succession en général, tel qu'expliqué par ceux qui en ont fait l'étude approfondie (Fuller, 1914 (27), Gleason, 1927 (28), Clements, 1928 (10), 1936 (11), Braun-Blanquet, 1932 (3)), suppose une amélioration





## CLIMAX

⊠ *Aceretum saccharophori laurentianum*

## QUASICLIMAX

● *Aceretum saccharophori betulosum*

◐ *Aceretum saccharophori tsugosum*

▽ *Aceretum saccharophori ulmosum*

♥ *Aceretum saccharophori quercosum*

⊙ *Aceretum saccharophori caryosum*

⋈ *Aceretum saccharophori nigroides*

## SOUS-CLIMAX

⊕ *Acereto-ulmetum laurentianum*

⊖ *Betuletum abietosum*

△ *Thujetum occidentale*

⊙ *Cratagetum boreale*

⋈ *Pinetum strobi*

■ *Betuletum laurentianum*

□ *Aceretum rubri*

## STADES PIONNIERS

▨ *Nupharetum variegati*

⊕ *Scirpetum elatum*

▨ *Calamagrostidetum canadensis*

▽ *Spiraeetum latifoliae*

▨ *Salicetum riparium*

▨ *Caricetum paludosum*

⊕ *Myricetum galeae*

▽ *Chamaedaphnetum calyculatae*

▨ *Kalmietum angustifoliae*

⊕ *Alnetum incanae*

▨ *Oenotheretum dumetorum*

▽ *Danthonietum spicatae*

⋈ *Festucetum rubrae*

⊕ *Agrostidetum stoloniferae*

⊕ *Trifolietum repentis*

▨ *Solidaginetum canadense*

FIG. 1. Carte généralisée des successions dans l'aire de la forêt décidue laurentienne

physique graduelle du site, une évolution topographique entraînant la maturation du sol (Jenny, 1941 (36)). De sorte que le milieu deviendra utilisable par des espèces de plus en plus exigeantes et de mieux en mieux capables d'en utiliser pleinement les ressources. C'est dans ce sens que l'on parle d'espèces *constructives*, *consolidantes* et *destructives* d'une association (Braun-Blanquet, 1932 (3)). Ainsi le *Pinus Strobus* est destructif pour le *Danthonietum* et les autres pelouses et prairies; il est consolidant pour le *Pinetum Strobi*. L'*Acer spicatum* est destructif pour l'*Alnetum incanae*, consolidant pour le *Betuletum abietosum* et constructif pour l'*Aceretum saccharophori betulosum*. La Fig. 1 tient compte de ce principe fondamental, l'association la plus franchement mésique et équilibrée, le climax, occupant le centre vers lequel toutes les autres convergent. Il importe peut-être d'insister sur le fait que ce diagramme ne figure pas des relations aréales. Comme Gleason (1927 (28)), l'a fait remarquer: "Areal zonation of vegetation does not constitute a sere, and is correlated with succession only in exceptional cases".

Ce qui ressort premièrement de cette représentation schématisée, c'est le fait que la succession suit cinq voies principales. Dans l'hydrosère, on distingue la sère de la plaine de débordement qui, à travers diverses formations ripariennes, atteint l'*Acereto-ulmetum laurentianum* (voir Dansereau, 1945 (18)), et la sère de la tourbière qui aboutit à un *Betuletum abietosum*. Dans la xérosère, à partir de danthonies ou d'onagres sur un sol nu, la transition se fait par divers types de prairies et de taillis vers le *Betuletum laurentianum* ou encore vers la pinède, le *Pinetum Strobi*. Il reste une cinquième voie, celle de la cédrière, atteinte à partir de stades pionniers humides ou secs.

La Fig. 2 montre sous une forme simplifiée ces cinq successions.

Le point central est occupé, bien entendu, par le climax, l'*Aceretum saccharophori laurentianum*. Il ne s'agit pas de redéfinir ici cette notion cardinale de la phytosociologie qu'on trouvera abondamment expliquée par Braun-Blanquet (1932 (3)), Clements (1936 (11)) et Cain (1939 (6)). Il suffit de nous rappeler que l'association-climax est celle qui, dans une aire donnée, marque le point terminal de l'évolution de la végétation dans les conditions les plus favorables, et qu'elle est déterminée par le climat ambiant et dure aussi longtemps que lui, alors que toutes les autres associations le sont par des facteurs plus immédiats, par exemple le sol et la topographie. De plus, on peut se représenter le climax comme l'unité phytosociologique qui utilise le mieux et le plus complètement toutes les ressources du milieu.

La relation de temps du climax avec les autres associations est à la base de beaucoup de discussions au sujet du monoclimate et des polyclimate, soit que l'on considère la durée du climax lui-même ou celle des sères. On pourrait poser la question de la façon suivante, en la réduisant à une formule. Soit *A*, la durée de l'évolution du premier stade pionnier au second; *B*, du second stade pionnier au troisième; *C*, du troisième au sous-climax; et *D*, du sous-climax au climax; soit *T* la durée du cycle climatique. Assurément, dans un bon nombre de cas, la relation peut s'exprimer ainsi:

$$(A + B + C + D) < T.$$

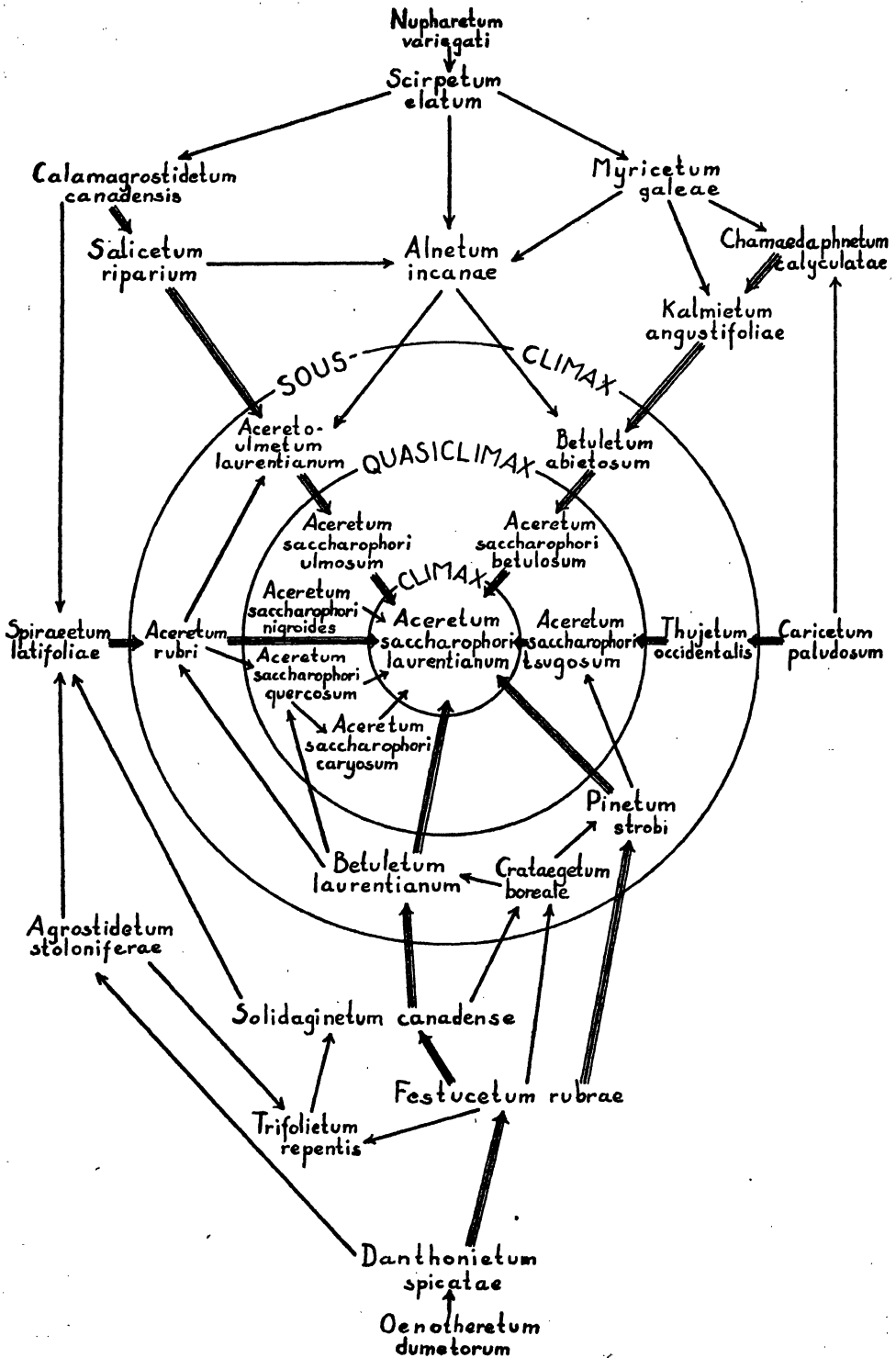


FIG. 2. Schéma des principales voies de succession dans l'aire de la forêt décidue laurentienne.

Autrement dit, il est possible à la sère entière de se développer au cours d'un même cycle climatique. On aura même souvent:

$$(A + B + C) < D < T.$$

Dans certains cas, toutefois, on aura:

$$(A + B + C + D) > T.$$

et puisque les divers stades de la sère sont de durée inégale, on peut avoir:

$$A > (B + C + D) \geq T.$$

Dans tous ces cas, il est clair que la définition du climax pourra dépendre de deux critères: la stabilisation progressive de la végétation dans les limites d'un climat de durée plus ou moins longue, et la loïsibilité de cette évolution dans toute son extension. Si l'on insiste sur la relation de temps, un stade pionnier (lichens de l'Arctique, par exemple) apparaîtra aussi stable que le climax lui-même. On pourra insister d'autre part sur le fait que, même si l'évolution de *A* à *D* n'est pas possible à l'intérieur d'un cycle climatique donné, *A* n'en tend pas moins vers *B*, *B* vers *C* et *C* vers *D*. De sorte que malgré les inhibitions de temps, auxquelles il faut ajouter les inhibitions topographiques, toute la végétation *tend* vers un équilibre unique, le climax.

C'est ainsi que l'entendent Braun-Blanquet (1932 (3)), Clements (1936 (11)), Cain (1939 (6)) et bien d'autres écologistes. Je ne prétends pas m'engager ici dans des considérations de temps, même pour fournir une échelle approximative de la durée des divers stades, mais plutôt insister sur l'enchaînement lui-même, l'ordre de la progression. Ainsi, sur les Figs. 1 et 2, la distance qui sépare chaque association du point central de convergence est proportionnelle à l'amélioration du site occupé: dans l'hydrosère, les associations qui jalonnent la succession marquent un assèchement progressif du sol, une amélioration du drainage et de l'aération; tandis que, dans la xérosère, elles enregistrent une augmentation du pouvoir de rétention d'eau des horizons supérieurs qui accompagne un enrichissement en matière organique et une différenciation plus marquée (Jenny, 1941 (36)). Fuller (1914 (27)) a bien démontré que le sol superficiel est une "région critique", puisque c'est là que se fait la régénération qui détermine la composition botanique et par conséquent le caractère même de la succession.

Nous distinguerons ici quatre niveaux dans l'évolution de la végétation, ainsi qu'il est indiqué dans la Fig. 2: les stades pionniers, les sous-climax, les quasi-climax et le climax. De plus, il sera question plus loin des proclimax et des disclimax. Mais, pour l'instant, nous nous attacherons à décrire les diverses étapes qui caractérisent la succession naturelle hors de l'intervention de l'homme (prisère). Par conséquent, les rétrogressions, les disclimax (subsère) et les reliques n'apparaissent pas comme tels dans les Figs 1 et 2.

### Les stades pionniers

L'initiation des diverses successions se fait à partir de trois points de départ principaux: l'eau, la pierre et le sol nu (argile ou sable). Or, les premières colonies que l'on rencontre sur chacun de ces substrats sont très diverses de

même que la rapidité relative de leurs transformations. Quel que soit le très grand intérêt que présentent ces phases initiales, il ne nous est possible, ici, que d'en esquisser la physionomie et la composition en des termes très généraux.

### L'HYDROSÈRE

Une longue progression se fait dans le milieu aquatique: selon les divers degrés de sédimentation et d'amélioration du sol lacustre, on voit apparaître des associations assez nettement différenciées: *Nupharetum*, *Vallisnerietum*, *Potamogetonum*, *Characetum* sont des communautés strictement aquatiques qui accomplissent en réalité un premier cycle. Le *Scirpetum* et le *Sagittarietum*, de même que certains *Caricetum* font la transition vers l'état palustre. Ces premiers stades manifestent sans doute une différenciation plus prononcée dans les milieux eutrophes et plus ou moins alcalins que dans la sère de la tourbière (Dansereau, 1945 (18)).

L'hydrosère comporte donc deux successions principales, celle des rivages et celle des tourbières. Il ne s'agit pas de développer ici dans toute son ampleur la zonation des rivages, mais seulement d'en esquisser les étapes les plus importantes, sans nous arrêter aux différences imposées par la texture et la réaction des sols.

Le *Nupharetum* sert de point de départ à l'hydrosère des rivages (Fig. 3). Le *Scirpetum* lui succède, qui peut, sur la fin de la saison de végétation, résister à une brève exondation (Dansereau, 1945 (18)). La première formation riparienne est le *Spartinetum* auquel fait suite le *Calamagrostidetum* où domine le *Calamagrostis canadensis* (Cléonique-Joseph, 1936 (12)). Cette bande de graminées constitue dans le nord-est américain une sorte de micro-prairie, en ce sens qu'elle présente des conditions analogues à celles qui prévalent dans la Prairie: sol mouillé au printemps (par l'inondation et l'infiltration) et très sec en été (par abaissement du niveau de l'eau et drainage excessif). Elle sert d'ailleurs d'abri à des espèces typiques de la Prairie qui font ici figure de reliques (de la période xérothermique?): *Sorghastrum nutans*, *Andropogon provincialis*, *Schizachyrium scoparium*, *Sporobolus heterolepis*, *Desmodium canadense* (Dansereau, 1944 (15)). La spirée (*Spiraea latifolia*), une espèce remarquablement euryhygrique et peu exigeante quant au sol, domine le stade suivant où figure assez souvent aussi le *Myrica Gale* dans les parties plus humides. Puis viennent les saules (*Salix* spp.) et le liard (*Populus deltoides* dans le sud-ouest du Québec, *Populus Tacamahacca* au nord-est). Cette zonation est fonction de la durée de l'inondation, et de l'ordre de résistance des espèces à ce facteur et à une déficience d'aération du sol. Toutes ces associations, qui couvrent la beine, la grève et la berge, si elles bénéficient des dépôts annuels de la crue, sont soumises en revanche à une certaine érosion, particulièrement aux eaux basses (Dansereau, 1945 (18)). Tandis que la plaine d'alluvion proprement dite (*floodplain*) ne se trouve guère exposée à ce processus et abrite un type forestier, l'*Acereto-ulmetum americanum*.

C'est souvent le *Nupharetum* qui sert également de point initial à l'hydrosère de la tourbière (Fig. 4). A cause de l'acidité ou de la pauvreté du milieu, le

*Calamagrostidetum* est remplacé ici par un *Caricetum* où dominent de grandes espèces comme les *Carex rostrata*, *C. lasiocarpa*, *C. lanuginosa*, etc. La zone suivante—où se rencontre fréquemment l'*Iris versicolor*—est dominée par le *Myrica Gale* auquel se mêle la spirée (*Spiraea latifolia*). Si la pente est assez

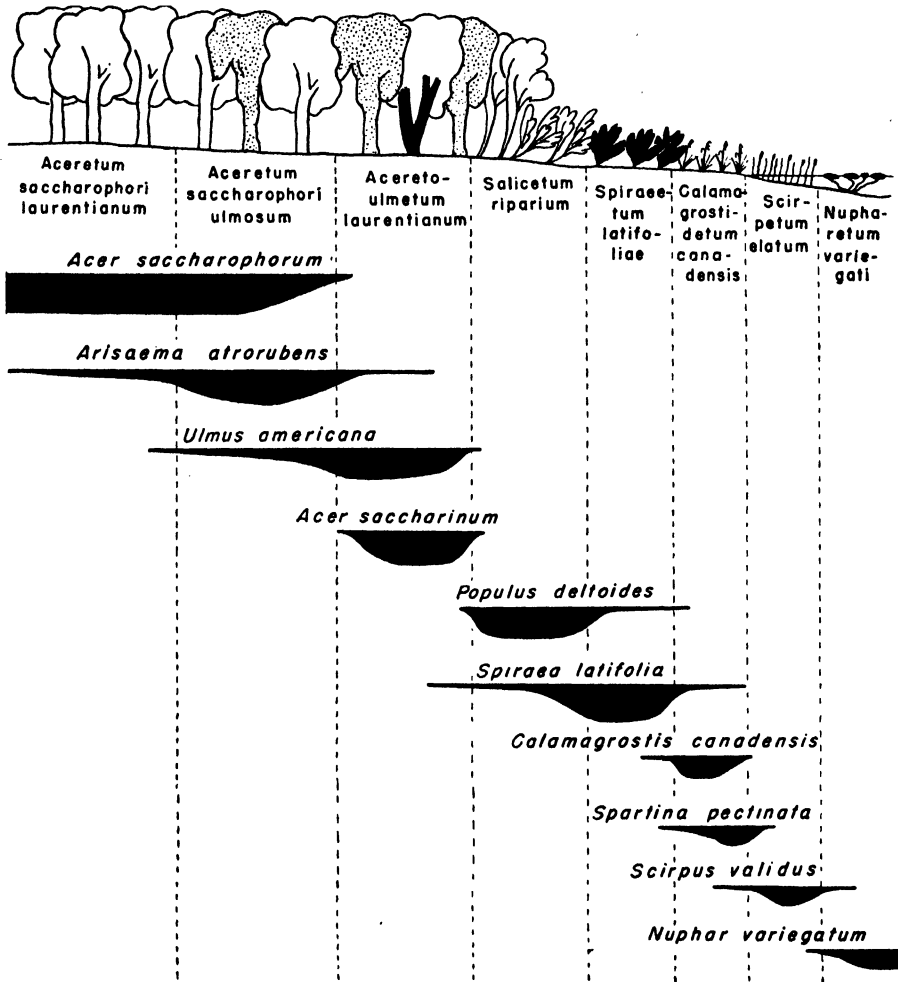


FIG. 3. Hydrosère des rivières.

douce pour causer des différences appréciables dans la durée des inondations, il peut se constituer derrière le *Myricetum* une mince bande d'*Andromeda glaucophylla*. Autrement, cette espèce sera dispersée par touffes dans les poches les plus humides de la zone suivante, le *Chamaedaphnetum*. Cette association, qui repose sur les sphaignes, n'admet presque aucune autre espèce que le *Chamaedaphne calyculata*, si ce n'est, sur les buttes un peu moins mouillées, le *Ledum groenlandicum*. A son tour, celui-ci occupe les biotopes les plus humides du *Kalmietum angustifoliae*. Cette formation relativement

plus stable, se compose d'un bon nombre d'espèces dont les plus importantes sont, outre les sphaignes, les *Kalmia angustifolia*, *Ledum groenlandicum*, *Vaccinium pennsylvanicum* et *Sarracenia purpurea*. Quelques arbres et arbustes sont piqués ici et là sur la surface plus ou moins élastique de la tourbe: *Larix*

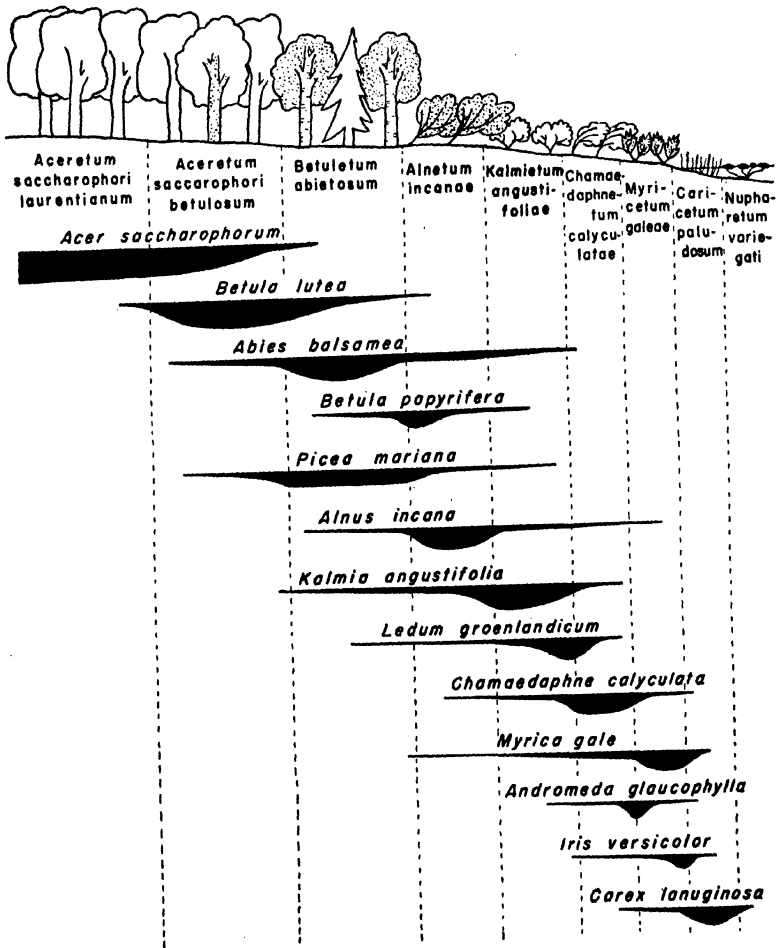


FIG. 4. Hydrosère de la tourbière.

*laricina*, *Picea mariana*, *Nemopanthus mucronata* et *Ilex verticillata*. Les aulnes (*Alnus incana*) deviendront éventuellement plus nombreux, au point de constituer des bosquets fermés (*Alnetum incanae*).

La présence de mésophytes comme les *Gaultheria procumbens*, *Trientalis borealis*, *Clintonia borealis*, *Maianthemum canadense*, ne se fait remarquer que vers la fin de ce stade lorsque la densité des arbres a augmenté. De toutes façons, l'hydrosère de la tourbière est une voie d'évolution plus commune pour

la forêt canadienne (climax: *Piceetum marianae* dans le nord du Québec) que pour la forêt décidue. En effet, dans la région (*Klimax Gebiet*) de l'*Aceretum saccharophori*, les tourbières font figure de reliques froides (voir diagramme dans Dansereau, 1944 (15)), et abritent une flore septentrionale dont la plupart des espèces herbacées qu'on vient de mentionner sont des représentantes. La bétulaie (*Betuletum abietosum*) qui les envahit trahit les mêmes affinités jusque dans sa strate arborescente.

Il reste la constitution de cédrières humides (*arbor vitae swamps*) à partir d'un *Caricetum* mouillé. Cette évolution est probablement, dans son ensemble, parallèle à la succession dans la tourbière. Il faudra connaître beaucoup mieux les sols de cette sère avant de pouvoir la définir même physionomiquement (voir plus loin, p. 257).

### LA XÉROSÈRE

Dans le milieu strictement terrestre, il est plus difficile que pour l'hydrosère de s'en tenir à l'ordre naturel ne mettant en cause que des plantes indigènes. L'action de l'homme sur les sols secs ou asséchés est partout présente et il est relativement malaisé de se représenter la succession primitive dans une nature vierge. L'utilisation que l'homme a faite de plusieurs des stades pionniers de la xérosère leur a donné à la fois une permanence et une importance écologique sans proportion avec leur rôle véritable dans la prisère. En décrivant les disclimax, plus loin, il faudra y revenir.

Les stades pionniers de l'hydrosère conduisaient à l'élaboration plus ou moins rapide de sous-climax arborescents: *Acereto-ulmetum laurentianum*, *Betuletum abietosum*, et *Thujetum occidentalis*. De même, la xérosère aboutit à deux sous-climax: la pinède (*Pinetum Strobi*) et la bétulaie (*Betuletum laurentianum*).

La xérosère de la pinède (Fig. 5) débute sur le sable pur et même sur la dune, colonisée au début surtout par des onagres (*Oenothera* spp.) et quelques graminées (*Ammophila breviligulata*, *Panicum capillare*, *Echinochloa pungens*). La danthonie vient ensuite, et sur bien des sortes de sols pauvres du reste (Frankton and Raymond, 1944 (26)). Une amélioration physique graduelle du sol, et particulièrement de sa structure et de sa capacité de retenir l'eau, i.e. d'utiliser les précipitations, entraînera l'invasion du *Danthonietum* par des espèces à peine plus exigeantes au début, telle la fétuque (*Festuca rubra*). Puis ce seront les agrostides (*Agrostis stolonifera*) euryhygriques, et enfin le pâturin bleu (*Poa pratensis*) et le trèfle blanc (*Trifolium repens*) (Frankton and Raymond, 1944 (26)). A ces quatre stades de pelouses à graminées courtes succède une prairie plus haute où dominent les verges d'or (*Solidago rugosa* et *canadensis*) et d'autres herbes à fort développement (*Verbascum Thapsus*, *Aster umbellatus*, *Veratrum viride*), et où s'introduiront de plus en plus les espèces ligneuses comme les spirées (*Spiraea latifolia* et *tomentosa*). Ce n'est guère avant ce stade que les aubépines (*Crataegus* spp.) ou les pins germeront et formeront un commencement de taillis.



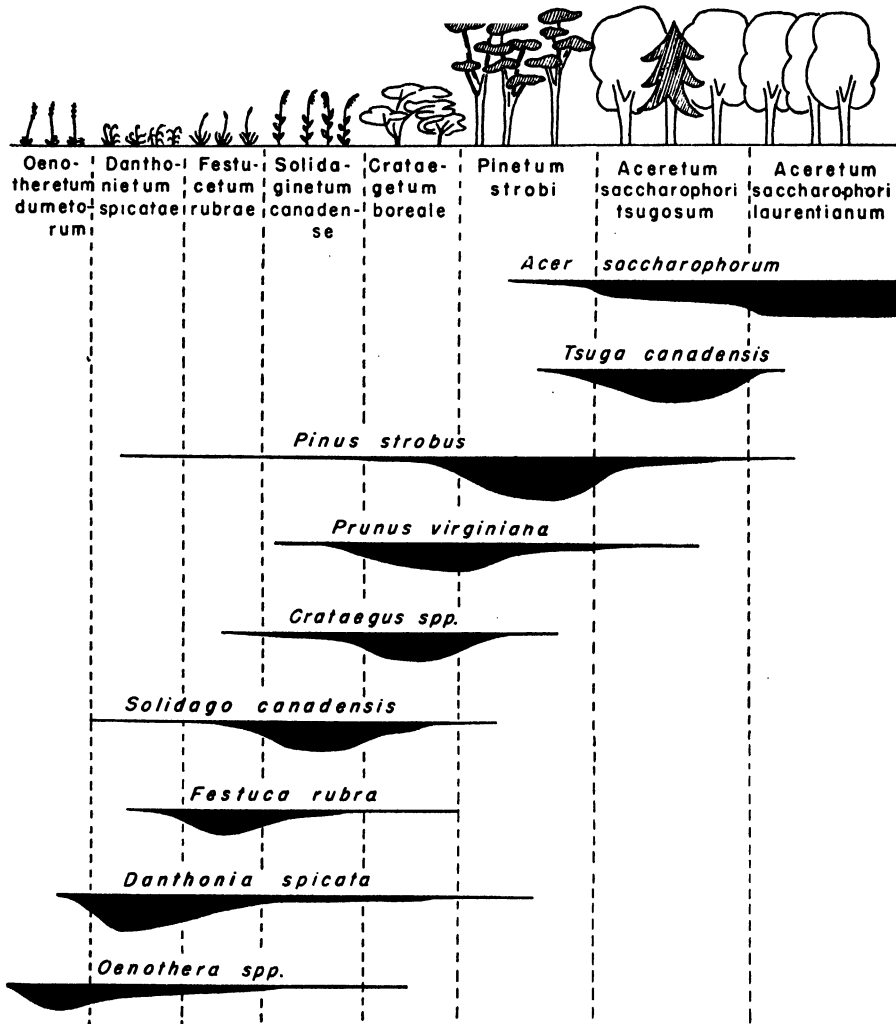


FIG. 5. Xérosère de la pinède.

La progression de la xérosère de la bétulaie est souvent tout à fait la même que ci-dessus (Fig. 6). Son point de départ, toutefois, n'est pas forcément un milieu siliceux. Ce peut être un sol argileux qui aura d'abord été colonisé par le trèfle d'odeur (*Melilotus alba*), les chénopodes (*Chenopodium album*), les amarantes (*Amaranthus retroflexus*) et les renouées (*Polygonum* spp.), et subséquentement envahi par le *Solidaginetum* mentionné plus haut.

### Les sous-climax

Pour la compréhension de notre sujet, l'étude des sous-climax est d'une importance plus immédiate: les conditions physiques et biologiques qui prévalent à ces stades relativement avancés des diverses sères auront une influence certaine sur la constitution du climax lui-même.

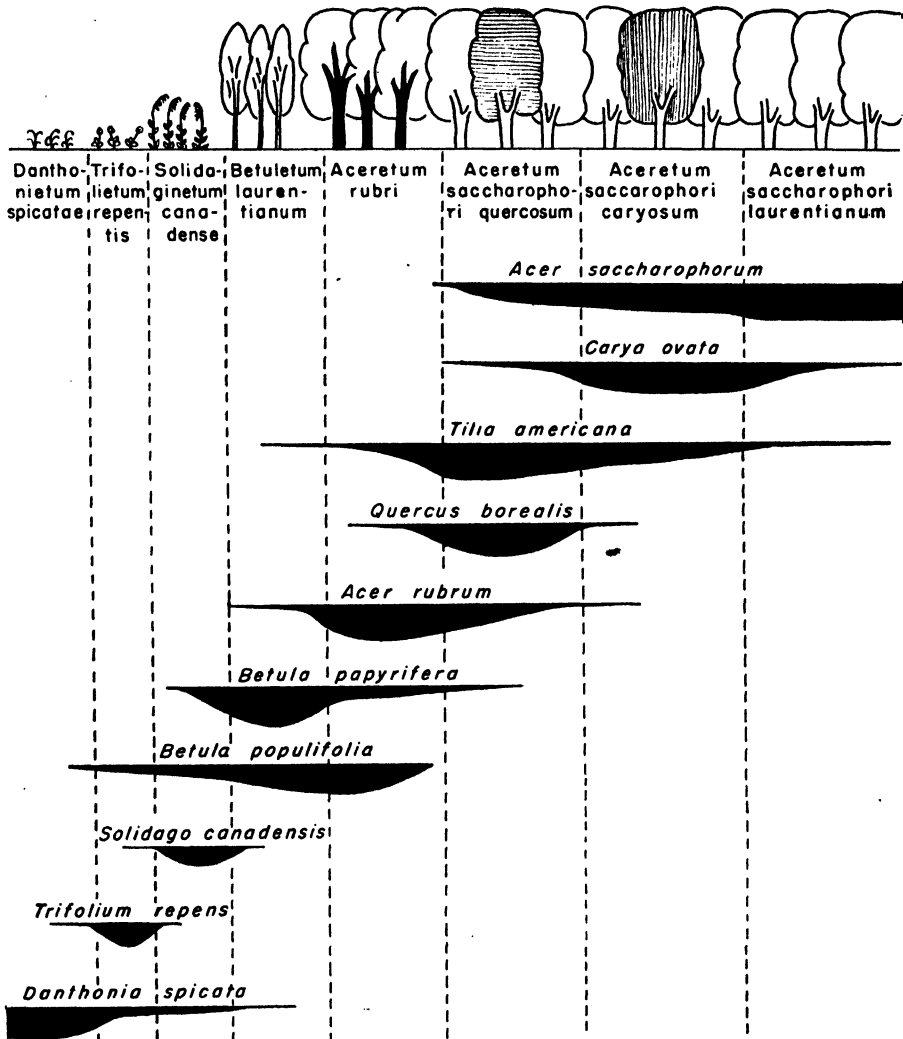


FIG. 6. Xérosère de la bétulaie.

Il n'existe malheureusement pas de définition très satisfaisante du sous-climax. Je désignerai ainsi les associations dans une sère quelconque qui ont la même structure que le climax (soit approximativement la même stratification et la même physionomie quant à l'abondance des individus de chaque forme biologique), mais dont la composition floristique se prête à une évolution ultérieure.

Les sous-climax sont donc des associations relativement mieux équilibrées que les stades pionniers précédents: il arrive souvent que leur durée soit considérable. Ceci s'explique par le fait que ce sont ici des communautés arborescentes, à plusieurs synusies présentant une évolution différentielle. Il est normal de s'attendre à un ralentissement de la progression qui se traduit par une apparente permanence. Bien entendu, la coupe, le feu, le pâturage,

l'inondation et d'autres agents résultant ou non de l'intervention humaine directe ou indirecte sont de nature à prolonger leur vie, jusqu'à les transformer quelquefois en disclimax.

Il sera question ici de sept sous-climax. Quelques-uns au moins affectent plusieurs faciès différents qui ne pourront être qu'esquissés. Je n'insisterai que sur les facteurs physiques et dynamiques qui conditionnent ces associations et sur les traits principaux de leur composition floristique. Il est à première vue évident que plusieurs d'entre eux devraient être subdivisés\*.

En décrivant ces sous-climax, les correspondances seront indiquées avec les types de la Society of American Foresters (1940 (48)). Cette classification plusieurs fois révisée a été établie par un comité de forestiers de grande expérience: R. M. Evans, R. D. Forbes, E. H. Frothingham, J. Kittredge jr., E. F. McCarthy, L. J. Pessin, J. N. Spaeth, L. Qyman et R. C. Hawley. Elle est purement descriptive, quoiqu'elle fasse intervenir d'une certaine façon un élément quantitatif, la dominance. Elle répond au besoin que l'on avait d'une classification physionomique et a le mérite d'être facilement utilisable. Sa valeur pour l'écologiste réside peut-être avant tout dans les indications du status dynamique de chaque type dans la sère, l'alignement général étant basé sur l'humidité du sol. D'autre part, il n'est fait aucune mention des espèces arbustives et herbacées, lesquelles sont généralement de meilleurs indicateurs de l'évolution récente de ce même sol et de sa productivité actuelle.

C'est pourquoi j'ai tenu à comparer aussi les types que je décris à ceux de Heimburger (1934 (32), 1941 (33)), établis selon les méthodes de Cajander (1926 (8)), qui attribue au sol l'influence prépondérante. Les types forestiers de ces auteurs sont décrits en tenant compte presque exclusivement de l'évolution de la strate herbacée (et quelquefois arbustive), et indépendamment de la coïncidence de cette phase avec une synusie arborescente de densité et de composition botanique définies†.

Quoiqu'elles tiennent compte des facteurs dynamiques, ces classifications ne me paraissent pas entièrement satisfaisantes, et il me semble que l'association telle que définie par l'école de Zurich et de Montpellier et par le sixième congrès international de Botanique à Amsterdam (1935) présente une image plus vraie de la végétation. Cette dernière définition considère l'association comme l'unité fondamentale—qu'il ne faudrait pas confondre avec l'unité la plus petite.

Les conceptions et les méthodes de Lippmaa (1939 (39)), en quelque sorte intermédiaires entre les précédentes, font bien ressortir la réalité de la synusie, la communauté de besoins, exigences et tolérances d'un petit nombre de plantes. D'autre part, l'extension géographique des *unions* nous fait un peu

\* Cela est vrai au point qu'on se demandera si ces sous-climax ne sont pas plutôt des alliances (sensu Braun-Blanquet, 1932 (3)) que des associations. Une discussion de la synécologie systématique des sous-climax dépasse cependant les cadres du présent mémoire et fera l'objet de travaux ultérieurs.

† "... on a basis of forest types it is possible to work out a biological basis of classification of localities essentially independent of species of trees." (Cajander, 1926 (8, p. 31).)

perdre de vue leurs affinités écologiques pour ne laisser subsister que leurs rapports floristiques.

Gleason (1936 (29)) s'est opposé à cette classification en alléguant qu'elle n'avait rien de dynamique, puisque les relations des individus d'une même strate sont très peu évidentes en comparaison de celles des individus des diverses strates. Autrement dit, une coupe horizontale reflète beaucoup moins l'utilisation différentielle des ressources du milieu et l'influence mutuelle qu'une coupe verticale.\*

Toutefois, il n'y a là aucune contradiction nécessaire: Lippmaa (1939 (39)) dit fort bien qu'il s'agit de niveaux d'intégration différents, et on ne contestera guère l'opportunité d'étudier la végétation à la fois ou successivement à la lumière de critères géobotaniques ou floristiques d'une part, et plus immédiatement écologiques et sociologiques (et par conséquent quantitatifs) d'autre part. Cain (1936 (5)), commentant les idées de Lippmaa, insiste sur les avantages qu'apporte au phytosociologue une connaissance exacte de chaque synusie, la stratification étant un des facteurs dynamiques les plus significatifs de l'habitat. Il importe, toutefois, de ne pas accorder à la synusie une valeur indépendante trop grande: Gleason (1936 (29)) cite plusieurs cas où les associations unistrates ne le sont que temporairement, quoiqu'il reconnaisse l'existence d'authentiques associations à une seule synusie. Si une union frutescente (v.g. *Nemopanthus-Ilex*), dans une tourbière, conserve une partie de sa cohésion, elle se trouve dans des conditions biologiques singulièrement altérées s'il se constitue éventuellement une synusie supérieure. De tels changements sont le plus souvent accompagnés de modifications considérables de la valeur de couverture et de la vitalité de chaque espèce. Le *Kalmia angustifolia*, en formation presque pure dans les tourbières à sphaignes, a un comportement, une densité et une vitalité tout autres sous la couverture légère des épinettes de la taïga hudsonienne.

Lippmaa (1939 (39)), Conard (1935 (13)), Cain et Penfound (1938 (7)) ont eux-mêmes admirablement analysé la structure écologique de forêts décidues en accordant une attention spéciale aux unions, mais en considérant toute la phytocénose. De pareils travaux sont de nature à faire ressortir l'ordre de dépendance des diverses synusies: les unes—v.g. l'union dominée par le *Kalmia latifolia* ou encore le *Taxus canadensis*—peuvent se trouver sous des synusies arborescentes de composition botanique différente (Cain, 1936 (5), Dansereau, 1946 (19)), mais offrent à peu près les mêmes caractères physiques; les autres—v.g. l'union à *Adiantum pedatum*—sont liées à une certaine composition botanique de la strate dominante; d'autres enfin, comme des unions de mousses ou de lichens sur les arbres, les pierres ou les souches,

\* "In a plant community, . . . the essential nature, the fundamental cause, the real ens of its existence, is non-areal, while its manifestation is distinctly areal, characterized by uniformity, extent, and boundary. . . . To bring together into one ecological unit all those plants with intersecting spheres of influence leads to a unit which not only possesses uniformity but is also a dynamic and genetic unit. To bring together into one unit only those plants which have exactly the same environment, namely, those which form a single synusia, leads to the divorce of cause and effect." (Gleason, 1936 (29), p. 448).

sont essentiellement discontinues et le plus souvent conditionnées par des agents physiques.

Or, ce qui peut-être importe le plus, c'est la continuité des synusies dans le temps. S'il est vrai que certaines se rencontrent—telles l'union à *Kalmia* ou à *Taxus* citées plus haut—sous diverses espèces d'arbres, ces diverses strates arborescentes ne sont-elles pas, à leur tour, génétiquement apparentées? De la sorte, la présence de la synusie en question peut être une *persistance* ou une *intrusion*, par conséquent un signe de l'évolution différentielle des synusies dans la sère.

La Fig. 7A fait voir schématiquement cette progression inégale, qui pourrait sans doute s'exprimer de plusieurs autres façons encore pour illustrer d'autres

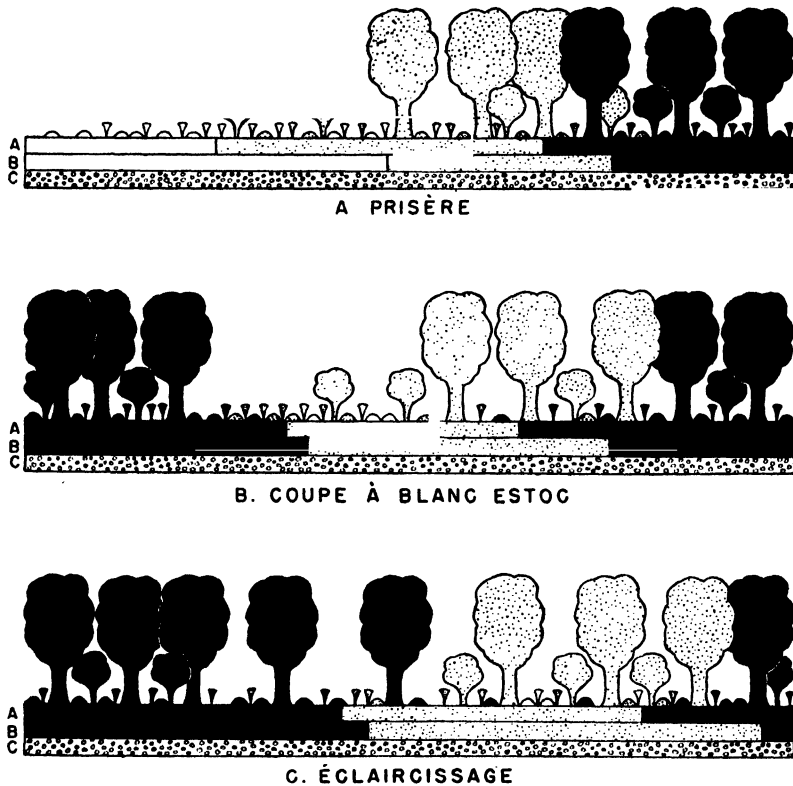


FIG. 7. Evolution différentielle des synusies et du sol dans la prisère (A) et la subsère (B et C).

combinaisons de retards et d'avances dans des conditions physiographiques diverses. Or, si l'on considère, en plus de la prisère, les subsères (ce que font toujours les forestiers, et entre autres Cajander), il devient immédiatement apparent que plusieurs conjonctions des facteurs physiques et biologiques vont se réaliser qui ne se présentent pas dans une nature vierge. Par conséquent, les corrélations successionnelles normales entre les unions peuvent être considérablement altérées, ainsi qu'il est indiqué sur les Figs 7B et 7C. Cajander

(1926 (8) ) n'a pas manqué de le souligner tout en insistant sur la valeur indicatrice des synusies herbacées.

Chaque union a donc un haut coefficient d'adaptation immédiate; mais il faut les considérer toutes pour bien se représenter à la fois la structure actuelle de l'association et son dynamisme. *La superposition des diverses mosaïques que sont les unions résulte de la somme totale des influences subies dans le passé, du pouvoir d'interférence des espèces et de leur adaptation à la conjonction actuelle des facteurs.*

Or, certains milieux demeurent physiquement assez hétérogènes—surtout les sous-climax et les disclimax—pour contenir dans un même habitat (homogène dans son ensemble) une juxtaposition d'unions différentes. La valeur indicatrice des espèces est souvent contradictoire, et l'utilisation par les plantes herbacées d'une faible portion du sol explique leur relative indépendance vis-à-vis de la composition botanique des strates supérieures. Le repérage de ces unions et de leur répartition me paraît très significatif, car elles ont des valeurs définies dans l'habitat lui-même au cours de la succession dont elles marquent les étapes.

On pourrait même, dans certains cas, considérer le *biotope*, le dernier échelon de l'intégration d'un être vivant dans son milieu. Ainsi, dans un ordre progressif de dépendance, on peut rencontrer dans une érablière: 1° un lichen limité à un *biotope*: l'écorce des érables; 2° une plante herbacée (*Trillium erectum*), limitée à une synusie ou *union*; 3° une plante arbustive (*Acer spicatum*) qui se rencontre, à différents âges, dans plusieurs unions; 4° une plante arborescente (*Fagus grandifolia*) qui atteint toutes les parties de l'*association*.

Je tenterai donc de considérer à la fois les conditions physiques (topographie, drainage, humidité, lumière, sol) et biologiques (formes biologiques, compétition, exigences et tolérance) en décrivant:

- a) les *associations*;
- b) les divers *faciès* ou dominances passagères ou localisées de certaines espèces ou de certaines unions, qui caractérisent les étapes de leur évolution ou des adaptations locales particulières, et donnent une physionomie à l'association elle-même;
- c) les synusies ou *unions* dont la superposition crée une structure caractéristique et dont la compénétration ou l'homogénéité indique le sens de la progression ou de la perturbation;
- d) les *biotopes* ou conditions très étroites (à l'intérieur d'une synusie ou union) qui permettent le développement d'une espèce-indice.

D'autre part, je tenterai de rattacher ces unités aux types correspondants de la Society of American Foresters (1940 (48) ) et de Heimburger (1934 (32), 1941 (33) ). Le Tableau I donne ces équivalences, de même que les types décrits par Westveld (1939 (54) ) et quelques autres auteurs cités dans la bibliographie. Le Tableau II présente un schéma des principales unions qui déterminent le faciès de ces associations.

TABLEAU I

COMPARAISON DES ASSOCIATIONS DÉCRITES ICI POUR LA RÉGION LAURENTIENNE AVEC LES TYPES FORESTIERS DÉFINIS PAR PLUSIEURS AUTEURS

NOM	SAF* 1940 (48)	Heimbürger 1934 (32), 1941 (33)	Westveld 1939 (54)	Autres
1. <i>Acereto-ulmetum laurentianum</i>	60			
2. <i>Betuletum abietosum</i>	21	Co-Ma Ba-O O-Co	Paper-birch-red-spruce -balsam-fir	Balsam-spruce associations (Stallard, 1929 (49) )
3. <i>Thugetum occidentalis</i>	24			<i>Thuja</i> consociation (Stallard, 1929 (49) )
4. <i>Crataegum boreale</i>				
5. <i>Pinetum Strobi</i>	9	V-G As-G As-Co	White pine	White pine consociation (Stallard, 1929 (49) )
6. <i>Betuletum laurentianum</i>	6	As-Ar	Paper birch Aspen	
7. <i>Aceretum rubri</i>	7, 26		Gray-birch-red-maple	<i>Aceretum rubri</i> (Cain and Penfound, 1938 (7) ) <i>Aceretum osmundaceum</i> (Conard, 1935 (13) )
8. <i>Aceretum saccharophori betulosum</i>	12	Vi-O (subtype) Vi	Sugar-maple-beech-yellow-birch	Hardwood type (Donahue, 1939 (24) )
9. <i>Aceretum saccharophori tsugosum</i>	10?	Ma-Vi Ba-O?	White-pine-hemlock?	Maple-beech-hemlock (Whitford, 1901 (55) )
10. <i>Aceretum saccharophori ulmosum</i>	13?	Aris Mi?		
11. <i>Aceretum saccharophori quercosum</i>	51	Oa	Red-oak-basswood-white-ash?	
12. <i>Aceretum saccharophori caryosum</i>	57?	Tr		
13. <i>Aceretum saccharophori nigroides</i>	57?			
14. <i>Aceretum saccharophori laurentianum</i>	57?			
15. <i>Aceretum saccharophori dennstaedtioides</i>				
16. <i>Aceretum saccharophori glyceriosum</i>				
17. <i>Aceretum saccharophori acerosum</i>				
18. <i>Aceretum saccharophori pascuorum</i>				

\* Society of American Foresters.

TABLEAU II

LES SOUS-CLIMAX, LEUR STRATIFICATION ET LEUR ÉVOLUTION. LES UNIONS SONT CARACTÉRISÉES PAR LES ESPÈCES DOMINANTES, SÉPARÉES DES CO-DOMINANTES PAR UN TRAIT. LA SUCCESSION A LIEU DANS LE SENS INDiqué PAR LES FLÈCHES, MAIS SANS CONCORDANCE ABSOLUE AUX DIFFÉRENTES SYNUSIES (VOIR FIG. 7)

Association	Union de la synusie arborescente	Unions de la synusie frutescente	Unions des synusies herbacées haute et basse
1 <i>Aceroidium-lumetum laurentianum</i>	<i>Acer saccharinum</i> <i>Ulmus americana</i> <i>Fraxinus nigra</i> <i>Salix nigra</i> <i>Acer rubrum</i>	<i>Ilex verticillata</i> <i>Rhamnus alnifolia</i> <i>Rhus radicans</i> <i>Cephalanthus occidentalis</i>	<i>Laportea canadensis</i> <i>Circaea latifolia</i> <i>Eupatorium perfoliatum</i> <i>Veratrum viride</i> <i>Symplocarpus foetidus</i> <i>Osmunda cinnamomea</i> <i>Pteris nodulosa</i> <i>Onoclea sensibilis</i>
2 <i>Betuletum abietosum</i>	<i>Betula papyrifera</i> <i>Abies balsamea</i> <i>Picea mariana</i> <i>Picea glauca</i> <i>Betula lutea</i>	<i>Ilex verticillata</i> <i>Nemopanthis mucronata</i> <i>Vaccinium corymbosum</i> <i>Viburnum cassinoides</i> <i>Viburnum lantanoides</i> <i>Lonicera canadensis</i> <i>Taxus canadensis</i>	<i>Linnaea borealis</i> <i>Cornus canadensis</i> <i>Copis groenlandica</i> <i>Dryopteris spinulosa</i> <i>Matantherum canadense</i> <i>Tridentalis borealis</i> <i>Trillium undulatum</i> <i>Tiarella cordifolia</i> <i>Lycopodium obscurum</i> <i>Lycopodium lucidulum</i> <i>Oxalis montana</i>
3 <i>Thujetum occidentalis</i>	<i>Thuja occidentalis</i>	<i>Alnus incana</i>	<i>Corallorhiza maculata</i> <i>Monotropa uniflora</i> <i>Pyrola elliptica</i> <i>Dalibarda repens</i> <i>Equisetum sibiricum</i> <i>Carex spp.</i> <i>Dicranum undulatum</i> <i>Calliergonella Schreberi</i>
4 <i>Crataegietum boreale</i>	<i>Crataegus spp.</i> <i>Amelanchier canadensis</i> <i>Betula populifolia</i>	<i>Prunus virginiana</i>	<i>Verbascum Thapsus</i> <i>Asclepias syriaca</i> <i>Danthonia spicata</i> <i>Hieracium aurantiacum</i> <i>Anemone nemorosa</i>



TABLEAU II—suite

LES SOUS-CLIMAX, LEUR STRATIFICATION ET LEUR ÉVOLUTION. LES UNIONS SONT CARACTÉRISÉES PAR LES ESPÈCES DOMINANTES, SÉPARÉES DES CO-DOMINANTES PAR UN TRAIT. LA SUCCESSION A LIEU DANS LE SENS INDiqué PAR LES FLÈCHES, MAIS SANS CONCORDANCE ABSOLUE AUX DIFFÉRENTES SYNUSIES (VOIR FIG. 7)—suite

Association	Union de la synusie arborescente	Unions de la synusie frutescente	Unions des synusies herbacées et	
	haute	basse		
5 <i>Pinetum Strobi</i>	<i>Pinus Strobus</i>	<i>Viburnum acerifolium</i>	<i>Vaccinium pennsylvanicum</i>	<i>Gaultheria procumbens</i> <i>Aster cordifolius</i>
				<i>Carex rugosperma</i> <i>Melampyrum lineare</i> <i>Danthonia spicata</i>
			<i>Pteridium latiusculum</i> <i>Rubus allegheniensis</i>	<i>Aster macrophyllus</i>
6 <i>Betuletum laurentianum</i>	<i>Betula papyrifera</i> <i>Populus tremuloides</i> <i>Prunus pennsylvanica</i> <i>Betula populifolia</i>	<i>Corylus cornuta</i>		<i>Corallorrhiza maculata</i> <i>Monotropa uniflora</i>
				<i>Pyrola elliptica</i>
				<i>Mitchella repens</i> <i>Dalibarda repens</i> <i>Chimaphila umbellata</i>
				<i>Trientalis borealis</i> <i>Trillium undulatum</i> <i>Lycopodium obscurum</i>
				<i>Aster macrophyllus</i> <i>Maianthemum canadense</i>
				<i>Danthonia spicata</i> <i>Antennaria neodioica</i> <i>Fragaria virginiana</i> <i>Lycopodium tristachyum</i>
			<i>Pteridium latiusculum</i> <i>Solidago</i> spp.	<i>Aster cordifolius</i> <i>Lycopodium clavatum</i>
			<i>Rubus</i> spp.	
			<i>Aralia nudicaulis</i>	<i>Trientalis borealis</i> <i>Trillium undulatum</i> <i>Lycopodium obscurum</i>
				<i>Aster macrophyllus</i> <i>Dryopteris spinulosa</i> <i>Maianthemum canadense</i>

TABLEAU 11—*fin*

LES SOUS-CLIMAX, LEUR STRATIFICATION ET LEUR ÉVOLUTION. LES UNIONS SONT CARACTÉRISÉES PAR LES ESPÈCES DOMINANTES, SÉPARÉES DES CO-DOMINANTES PAR UN TRAIT. LA SUCCESSION A LIEU DANS LE SENS INDiqué PAR LES FLÈCHES, MAIS SANS CONCORDANCE ABSOLUE AUX DIFFÉRENTES SYNUSIES (VOIR FIG. 7)—*fin*

Association	Union de la synusie arborescente	Unions de la synusie frutescente	Unions des synusies herbacées	
			haute	et basse
7 <i>Aceretum rubri</i>	<i>Acer rubrum</i> <i>Betula populifolia</i> <i>Betula papyrifera</i> <i>Ulmus americana</i> <i>Fraxinus nigra</i>		<i>Carex trinita</i>	<i>Equisetum sylvaticum</i>
			<i>Osmunda cinnamomea</i>	→
			<i>Pteritis nodulosa</i>	→
			<i>Onoclea sensibilis</i>	→
			<i>Impatiens biflora</i>	←
			<i>Veratrum viride</i> <i>Symplocarpus foetidus</i> <i>Eupatorium perfoliatum</i>	←

## 1. ACERETO-ULMETUM LAURENTIANUM (SAF no 60)

C'est la forêt de la plaine de débordement, dont le sol s'enrichit chaque année d'un dépôt. L'inondation cause donc un renouvellement perpétuel de l'horizon A et maintient les racines dans l'eau pendant une période assez longue. La structure du sol elle-même doit être améliorée chaque année par les espèces herbacées et ligneuses qui la travaillent après le retrait des eaux. Les difficultés de pénétration de l'air dans ce sol et une couverture arborescente dense qui empêchent son évaporation superficielle sont sans doute les deux facteurs les plus importants.

Dans l'union supérieure, le principal indicateur est l'*Acer saccharinum*, toujours fidèle à cette association dans l'aire géographique en question. L'*Ulmus americana* y est aussi dominant, tandis que le *Salix nigra*, le *Fraxinus nigra*, l'*Acer rubrum* y assument des proportions variables. L'étage arbustif est occupé par le *Rhus radicans*, souvent très développé, et parfois par l'*Ilex verticillata*, le *Rhamnus alnifolia*. Dans des conditions de drainage plus difficile, il peut se constituer un faciès à *Cephalanthus occidentalis*. Les herbes constituent une mégaphorbiée dont la densité est en fonction inverse du pourcentage de couverture arborescente. Ce sont surtout des nitrophiles comme le *Laportea canadensis*, l'*Eupatorium perfoliatum*, le *Circaea latifolia* ou en tout cas des mégaphylles comme le *Veratrum viride* et le *Symplocarpus foetidus*, et aussi des fougères: *Onoclea sensibilis*, *Osmunda cinnamomea* et *Pteritis nodulosa*.

Il est assez remarquable que cette association se prolonge loin vers le nord, le long de la rivière Gatineau, à l'ouest de Mont-Laurier (Marie-Victorin et Rolland-Germain, 1942 (44)), accomplissant encore là son rôle de sous-climax vis-à-vis des érablières devenues plus rares et disséminées parmi les sapinières. D'autre part, vers le sud, elle a des points de contact avec l'*Aceretum rubri* (voir plus loin, et aussi la Fig. 1) et par conséquent avec le type SAF 26.

L'*Acereto-ulmetum laurentianum* constitue un serclimax, en ce sens qu'en bordure d'un même cours d'eau il doit sa permanence à un lent déplacement à mesure que les conditions de drainage s'améliorent. Ce complexe phytosociologique comporte plusieurs vicariants, dont le *Platanetum occidentalis* (SAF 59) à l'ouest des Appalaches surtout et le *Populetum balsamiferae* vers le nord-est.

## 2. BETULETUM ABIETOSUM (SAF 21, HEIMBURGER CoMA)

Comme il a été dit plus haut, la sère de la tourbière tout entière présente des conditions froides et humides qui favorisent les espèces nordiques. Cette sère, plus au nord, aboutit au *Piceetum marianae* (Parc National des Laurentides) ou au *Piceetum glaucae* (Gaspésie); vers le sud, en altitude, elle tend vers le *Piceetum rubentis*. Dans la partie de l'aire de la forêt décidue que compénètre la sapinière (dans les Laurentides, par exemple), elle se termine fréquemment par l'un de ces trois climax du biome de la forêt canadienne.

Le *Betuletum abietosum* est donc caractérisé par le *Betula papyrifera* qui a envahi la tourbière en voie d'assèchement. Le sapin (*Abies balsamea*) est un

élément d'autant plus abondant que le milieu se réchauffe mal (dans un bas-fond avec drainage de courants d'air froids) et que l'association est âgée, sa reproduction étant meilleure que celle du bouleau. Les épinettes, quoique fréquentes, n'atteignent jamais une abondance notable. Le merisier (*Betula lutea*) est généralement présent et il remplace peu à peu le bouleau blanc. Au début, il peut y avoir quelques individus de *Pinus Strobus* et de *Populus tremuloides*.

L'étage arbustif comprendra d'abord une union à *Nemopanthus mucronata*, *Ilex verticillata*, *Vaccinium corymbosum*, *Viburnum cassinoides*, qui sera peu à peu remplacée par une autre à *Viburnum lantanoides* et *Lonicera canadensis* ou à *Taxus canadensis*.

L'union herbacée contiendra au début nombre d'espèces boréales, comme le *Linnaea borealis* var. *americana*, le *Cornus canadensis*, le *Coptis groenlandica*, qui le céderont peu à peu à des espèces d'affinités moins nettement boréales, comme les *Maianthemum canadense*, *Trientalis borealis*, *Lycopodium lucidulum*, *Trillium undulatum* et *Tiarella cordifolia*. Le *Dryopteris spinulosa* et l'*Oxalis montana* seront toujours des éléments caractéristiques.

Les sphaignes persisteront à l'état d'îlots dans les dépressions les plus mal drainées; cependant que des mousses plus mésophiles (*Calliergonella Schreberi*, *Hylocomium splendens*, *Hypnum Crista-castrensis*) occuperont des biotopes moins humides.

Ce sous-climax est extrêmement répandu dans la vallée du Saint-Laurent, surtout à partir de quelque 500 pieds d'altitude et dans l'écotone, ou marche, où se fait le contact de la forêt canadienne et de la forêt décidue (voir Dansereau, 1944 (15, 16)). On peut le considérer comme une sorte de point de bifurcation entre deux climax, puisqu'il sera envahi, selon la topographie et le microclimat par des espèces qui préludent soit au *Piceetum marianae*, soit à l'*Aceretum saccharophori*. En l'assimilant au type *Cornus-Maianthemum* de Heimburger, on le situerait plutôt dans la sère du *Piceetum* où les espèces septentrionales mentionnées plus haut gagneront de plus en plus de terrain. Tandis qu'en le rapprochant des types *Bazzania-Oxalis* ou *Oxalis-Cornus*, l'évolution vers un site à meilleur drainage et à réchauffement plus rapide est indiquée.

À l'état de maturité, donc, le *Betuletum abietosum* abritera un tapis de mousses diminué et discontinu, le sol s'étant asséché, et les espèces boréales auront régressé au profit d'éléments plus mésiques (par exemple, ceux du *Betuletum laurentianum*).

La grande extension géographique de ce sous-climax est une des principales raisons pour lesquelles on a établi une zone dite des "bois mêlés" (Ab-Yberg, 1931 (1), Marie-Victorin, 1935 (42), Halliday, 1937 (31)), dont les espèces constituantes énumérées plus haut ne sauraient former un peuplement permanent.

### 3. THUJETUM OCCIDENTALIS (SAF 24)

La cédrière pose de nombreux problèmes dans nos régions. Pour bien comprendre sa position écologique, il faudrait pouvoir interpréter correctement les exigences du *Thuja occidentalis*. On est tenté d'écrire des *Thuja*

*occidentalis*, tant cette espèce se présente dans des habitats apparemment divers.

Le cèdre n'est nulle part plus beau qu'en Gaspésie. Dans certains vieux *Piceetum* intacts, on trouve des individus dépassant 75 pieds de hauteur et trois pieds de diamètre. Ces arbres sont en pleine forêt mésophile. Rien de tel dans la plaine du Saint-Laurent où le cèdre est restreint aux collines calcaires sèches ou aux dépressions humides, et n'atteint guère 30 pieds de hauteur ni un pied de diamètre. Marie-Victorin (1935 (42)) définit ses exigences de la façon suivante: "Dans la région de Montréal la présence de bosquets de *Thuja* indique le plus souvent un sous-sol constitué de calcaires ordoviciens. Dans les Laurentides, où les roches sont acides, le *Thuja* se tient au bord des lacs; il semble que l'eau, en lavant continuellement les racines, entraîne les produits acides, et maintienne le pH au niveau voulu."

Dans les Laurentides le cèdre occupe maints habitats où cette dernière explication paraît d'autant moins plausible qu'il est immédiatement entouré d'oxylophytes bien caractérisées comme les *Vaccinium*, *Kalmia*, *Ledum*, etc. Marie-Victorin et Rolland-Germain (1942 (44)) décrivent eux-mêmes des cédrières qui ne sont ni plus ni moins que des tourbières à sphaignes abritant quelques *Thuja*.

S'il ne s'agit pas de races physiologiques distinctes de *Thuja occidentalis*, et qu'on cherche un commun dénominateur à ces adaptations contradictoires, il faudra peut-être considérer la résistance plutôt que les exigences de l'espèce. Son succès dans des habitats aussi divers ne serait-il pas fonction de sa tolérance d'une aération déficiente des racines?

Sur les terrains secs, dans la plaine du St-Laurent, le *Thuja* joue un rôle dévolu plus au nord à l'épinette blanche (*Picea glauca*) qui germe bien sur un sol minéral mais qui rencontre là un climat trop chaud; et plus au sud au *Juniperus virginiana* (SAF 46) qui s'arrête en deçà d'un climat trop froid (atteint ses limites près d'Ottawa et sur les bords du lac Champlain).

Le *Thujetum occidentalis* devient éventuellement très dense, et n'admet à peu près aucune végétation herbacée, sauf peut-être le *Corallorrhiza maculata* et le *Monotropa uniflora* et quelques-unes des espèces du sous-bois du *Pinetum Strobi* (*Dalibarda repens*, *Pyrola elliptica*, *Equisetum scirpoides*).

Sur un sol humide, toutefois, il peut se constituer une union d'*Alnus incana* et, la lumière n'étant pas trop réduite, des *Carex* et quelques coussinets de *Calliergonella Schreberi*, *Dicranum undulatum*, etc.

#### 4. CRATAEGETUM BOREALE

Les taillis d'aubépines sont un sous-climax qui dépend plus ou moins immédiatement de l'homme et comme tels seraient peut-être mieux nommés disclimax. Marie-Victorin (1938 (43)) croit que les *Crataegus* sont venus du sud au cours de la période historique. Il ne fait aucun doute que leur dissémination a été grandement favorisée par l'éclaircissage et la suppression de la forêt primitive. Que leur grande mutabilité résulte ou non de leur libération

de la compétition des mésophytes, les cenelliers sont assurément un important élément floristique de l'aire Hudson-Champlain-St-Laurent, mais ils ne font pas ordinairement partie de la prisère.

Les conditions favorables aux aubépines sont: l'abondance de lumière et un sol assez bien drainé. Par ailleurs, ces arbustes résistent très bien à un sol pauvre ou mal aéré. Surtout, ils tiennent à peu près indéfiniment contre un facteur biotique, le pâturage.

Les associés des aubépines sont très variables, étant donné la dégradation plus ou moins avancée du sol. Ce sont toutefois des héliophiles surtout et des mauvaises herbes: *Betula populifolia*, *Amelanchier canadensis*, *Prunus virginiana*, *Verbascum Thapsus*, *Danthonia spicata*, *Taraxacum officinale*, *Hieracium aurantiacum*, *Antennaria neodioica*, *Anaphalis margaritacea*, etc.

##### 5. PINETUM STROBI (SAF 9; HEIMBURGER V-G, As-G, As-Co)

L'extension géographique des formations de pin blanc est très considérable et pose des problèmes qui ne peuvent être abordés dans les cadres du présent travail. Les idées de Nichols (1935 (45)), de Halliday (1937 (31)), de Dice (1938 (23)) et d'autres biogéographes seront discutées dans un autre mémoire où il sera question de tracer les frontières des diverses associations-climax que l'on rencontre à l'intérieur du biome de la forêt décidue d'une part et du biome de la forêt canadienne d'autre part. La question de la compénétration des climax (Dansereau, 1944 (16)) ne saurait être résolue sur une base exclusivement floristique ou faunistique. Il me semble précisément que les variations géographiques de l'adaptabilité écologique sont de toute première importance à ce sujet. C'est pourquoi une connaissance des étapes principales de la succession me paraît nécessairement préalable à une discussion des biomes et de leur répartition.

Il se peut que les bois de pin blanc ou un mélange de pin blanc et de pruche (*Pineto-tsugetum*) soient le climax de certaines régions des Grands Lacs (Weaver and Clements, 1938 (53)), particulièrement au voisinage immédiat de la Prairie (Stallard, 1929 (49)).

Or, le *Pinetum Strobi*, tel que constitué dans la Vallée moyenne du St-Laurent, couvre rarement de grandes étendues. Il occupe les dépôts alluviaux sablonneux et pauvres, et sa stabilité est très douteuse, en ce sens qu'il se déplace continuellement, à la manière d'autres serclimax (voir l'*Aceretoulmetum* ci-dessus), avec l'avance ou le recul des bandes de végétation qui composent la zonation.

La résistance notoire du pin blanc à la sécheresse ne le condamne pas exclusivement aux terrains toujours secs: on le trouve jusqu'en marge des toubières, particulièrement sur des sols qui s'assèchent beaucoup en juillet-août. Dans les Etats-Unis du Centre-Nord et dans l'état de New-York, il n'est pas rare de le rencontrer dans des habitats humides ou tout au moins humides au début de la saison. C'est l'habitat par excellence du *Rhus vernix* (Lindsey, 1932 (38)), très rare dans la plaine de Montréal (Laprairie, Ste-Victoire). Il semble d'ailleurs que ce *Pinetum paludosum* près de sa limite

nord contienne beaucoup plus d'érable rouge et de merisier que de pin, ces deux espèces pouvant mieux y accomplir leur cycle. A Long Island, c'est plutôt le pin jaune (*Pinus rigida*) que l'on trouve en pareil milieu (voir plus loin sous *Aceretum rubri*; aussi, Cain and Penfound, 1938 (7)).

Le pin blanc est donc généralement confiné ici aux terrains sablonneux secs. Au cours de l'évolution de cette association, toutefois, il se produit une succession de synusies arbustives et herbacées en relation avec le sol, de sorte qu'on distinguera plusieurs étapes caractérisées par des faciès bien reconnaissables.

*Faciès à Vaccinium-Gaultheria.* Au début, sur un sol purement minéral et excessivement drainé, et sous une couverture arborescente encore clairsemée, le *Viburnum acerifolium*, le *Vaccinium pennsylvanicum*, le *Danthonia spicata*, l'*Aster cordifolius*, le *Carex rugosperma*, le *Lycopodium tristachyum* s'étendent en formation plus ou moins ouverte et peu à peu colonisée par le *Gaultheria procumbens*, le *Melampyrum lineare* (type *Vaccinium-Gaultheria* de Heimburger).

*Faciès à Aster-Pteridium.* Des espèces à plus fort développement foliaire, à feuillage décidu plus enrichissant pour le sol marquent l'étape suivante: *Aster macrophyllus*, *Pteridium latiusculum* et *Rubus allegheniensis* (type *Aster-Gaultheria* de Heimburger).

*Faciès à Monotropa.* Au cours de l'évolution des biotopes précédents, la couronne des arbres se referme, et les espèces herbacées caractéristiques disparaissent peu à peu. Il peut se produire une période de stagnation à peu près complète: les précipitations d'été n'atteignent pratiquement pas le sol. Celui-ci est dans une ombre totale. Le niveau superficiel est fait d'aiguilles peu décomposées et cette litière n'absorbe pratiquement pas d'eau, ne peut la retenir et se réchauffe excessivement. A peu près aucune germination n'est possible dans ces conditions et seule la chute d'un arbre (de 150 à 200 ans?) améliorera les conditions et permettra un pas de plus à la succession. Des saprophytes comme le *Monotropa uniflora* et le *Corallorrhiza maculata* sont les espèces les plus susceptibles de se maintenir à ce stade.

*Faciès à Corylus.* La pinède adulte, c'est-à-dire celle dont l'amélioration du sol a évolué parallèlement à la croissance des arbres à cause d'un drainage moins excessif ou d'une meilleure texture du sol au début, contient encore un nombre variable des espèces mentionnées plus haut (sauf celles du faciès à *Vaccinium-Gaultheria*), et particulièrement l'*Aster macrophyllus*, mais elle est caractérisée surtout par la présence d'éléments plus mésiques. Il se forme une union à *Corylus cornuta*, et une union herbacée à *Maianthemum canadense*, *Pyrola elliptica*, *Mitchella repens*, *Lycopodium obscurum*, *Trillium undulatum*, *Chimaphila umbellata*, *Dalibarda repens* (type *Aster-Corylus* de Heimburger).

Deux autres types de pinède se rencontrent dans notre aire, dominés par le pin rouge (*Pinus resinosa*) et le pin gris (*Pinus Banksiana*). Les types *Cornus-Pyrola* et *Vaccinium-Myrica* de Heimburger se rattachent à ces

formations respectivement plus xérique et plus nordique que le *Pinetum Strobi*. Toutefois, le *Pinetum resinosa* (SAF 3) et le *Pinetum Banksianae* (SAF 1), dans l'aire en question, font plutôt figure de reliques et ne seront pas décrites. Là où elles existent, elles remplacent le stade le plus primitif de la pinède blanche (SAF 9).

#### 6. BETULETUM LAURENTIANUM (SAF 6; HEIMBURGER AS-AR)

On pourra facilement juger par sa position et son extension dans la Fig. 1 de l'importance de cette association dans les diverses successions. En réalité, il faut subdiviser considérablement et reconnaître nombre de faciès écologiques, et même d'associations distinctes.

Dans les endroits les plus secs, on trouvera un *Populetum tremuloidis* (SAF 4) ou un *Prunetum pennsylvanicae* (SAF 5), surtout après les feux. On rencontrera aussi, et très souvent dans le sud de notre aire, des formations pures de *Betula populifolia*, évoluant vers l'*Aceretum rubri* (voir le numéroc suivant) à partir de stades pionniers qui peuvent être secs ou humides.

Mais, d'une façon générale, ce sera plutôt au *Betula papyrifera* que sera dévolu le rôle dominant, et les espèces mentionnées plus haut seront subdominantes ou même seulement occasionnelles.

Sur les sites les plus humides ce type se rapprochera du *Betuletum abietosum* et sur les plus secs, du *Pinetum Strobi* décrit plus haut, et abritera des arbustes et herbes caractéristiques de ces associations. Il a aussi des affinités avec le *Thujetum* qui pourra l'envahir, surtout si le drainage est déficient.

Le caractère qui lui est propre, toutefois, lui sera donné par l'un ou l'autre des faciès suivants ou par une mosaïque de deux ou plusieurs d'entre eux.

*Faciès à Antennaria.* Sur des sols très secs ou très épuisés, en présence d'une synusie arborescente encore claire (couverture de moins de 40%), la végétation herbacée comprendra les *Fragaria virginiana*, *Antennaria neodtoica*, *Danthonia spicata*, *Lycopodium tristachyum*.

*Faciès à Aster cordifolius.* Sur un sol un peu amélioré, des espèces plus tolérantes de l'ombre les remplaceront: *Lycopodium complanatum*, *L. clavatum*, *L. obscurum*. Une seconde union herbacée apparaîtra composée de l'*Aster cordifolius*, de divers *Solidago* et du *Pteridium latiusculum*. Celui-ci pourra s'étendre éventuellement en formation presque pure (faciès *Aster-Pteridium* cité plus haut) jusqu'à ce que la densité de la voûte arborescente augmente considérablement, soit pour atteindre une couverture de plus de 50%.

*Faciès à Rubus.* Dans un bois clair, les *Rubus allegheniensis* et *R. idaeus* pourront former des fourrés plus ou moins impénétrables pour les autres espèces.

*Faciès à Aralia nudicaulis.* En présence d'un sol suffisamment humide et riche, l'*Aster macrophyllus* et l'*Aralia nudicaulis* accaparent une partie considérable de l'espace (type *Aster-Aralia* de Heimbürger). D'autres espèces mésiques mais peu exigeantes, les accompagneront, mais en nombre restreint et avec une valeur de couverture habituellement très basse: *Lycopodium obscurum*, *Trientalis borealis*, *Maianthemum canadense*.



*Faciès à Maianthemum-Dryopteris.* Là où les fluctuations d'humidité du sol ne se font presque plus sentir, autrement dit où le drainage est plus ou moins régularisé, on trouvera le *Maianthemum canadense* et le *Dryopteris spinulosa*.

7. ACERETUM RUBRI (SAF 7; CAIN AND PENFOUND, 1938 (7) )

La composition botanique de cette association peut varier beaucoup. A l'instar de la précédente, on peut la subdiviser, d'autant plus que ses contacts dans l'hydrosère et dans la xérosère sont nombreux (voir Figs 1 et 2) et supposent des origines et des évolutions diverses. Or, ses affinités les plus marquées sont avec le numéro précédent et avec l'*Acereto-ulmetum laurentianum*.

Ce sous-climax se constituera surtout dans les endroits à drainage lent ou irrégulier. Il correspond à un stade d'immaturité du sol où la couche de matière organique est encore peu développée.

Sur les alluvions sablonneuses, l'*Acer rubrum* sera en compagnie du *Betula populifolia*; en milieu plus mésique, la synusie arborescente ressemblera plus ou moins à celle du *Betuletum laurentianum* et comportera un certain pourcentage de *Betula papyrifera*; ailleurs l'*Ulmus americana* et les *Fraxinus americana* et *nigra* seront présents (SAF 26 et *Aceretum rubri* de Cain et Penfound, 1938 (7) ).

Dans des conditions aussi hétérogènes, un bon nombre des unions déjà décrites pour le *Pinetum Strobi* et le *Betuletum laurentianum* pourront être réalisées.

Quelques faciès, toutefois, sont plus particuliers à l'*Aceretum rubri* ou n'ont pas encore été décrits.

*Faciès à Carex crinita.* Sur un sol bossué, inégal, il se constitue des dépressions communiquant entre elles par des rigoles. Dans le cas où ces dépressions ne s'assèchent pas tout-à-fait l'été, il se forme un sol relativement riche mais peu aéré où domine le *Carex crinita*, avec l'*Equisetum sylvaticum*, le *Pteretis nodulosa*.

*Faciès à Osmunda cinnamomea.* Là où les dépressions mentionnées plus haut s'assèchent quelque peu, sans que l'évaporation soit très forte (à cause de la densité de la synusie arborescente), c'est l'*Osmunda cinnamomea* qui domine (*Aceretum osmundaceum* de Conard, 1935 (13) ).

*Faciès à Onoclea sensibilis.* Toujours dans les dépressions, longuement inondées au printemps, et là où la végétation ne débute guère avant le mois de juin, c'est l'*Onoclea sensibilis* qui domine d'abord. Bientôt les espaces demeurés libres sont comblés par l'*Impatiens biflora*. Cette formation ne se constitue probablement pas sur un sol trop acide.

*Faciès à Veratrum viride.* Si la couverture arborescente est inférieure à 50%, le *Veratrum viride* pourra former des colonies presque pures sur un sol mouillé au printemps, même si l'assèchement estival est assez prononcé. On trouvera là également le *Symplocarpus foetidus*, l'*Eupatorium maculatum*.

### Les quasiclimax

Chacun des sept sous-climax brièvement caractérisés plus haut est susceptible d'être envahi directement par le climax climatique, l'*Aceretum saccharophori*. Rappelons que pratiquement tous les télescopages sont possibles dans une succession quelconque, chacun d'entre eux correspondant à une accélération dans les processus d'évolution de la végétation. N'oublions pas non plus l'avance différentielle des synusies dont il a été question plus haut (Fig. 7).

Cependant, le contraire du télescopage peut se produire, à la fois dans le temps et dans l'espace: il est bien connu qu'une zonation comportant un grand nombre de bandes nettement caractérisées n'est possible qu'à la faveur de gradients plus ou moins insensibles qui offrent des conjonctions différentes de facteurs physiques favorables à toute une gamme d'espèces ayant des exigences et des tolérances différentes mais très voisines. De même, une certaine lenteur de l'évolution dans le temps permet à des paliers de se constituer au cours d'une sère. Si ce retardement affecte le stade sub-terminal (la fin du sous-climax), il influe sur le climax lui-même auquel il peut imposer des variantes qui se traduiront par un faciès particulier. De la sorte, on aura affaire à un *climax légèrement inhibé, qui ne réalise pas toutes les possibilités normalement permises par le climat local*, et qu'on appellera *quasiclimax*.

Ces retardements seront de deux sortes, et serviront bien à faire ressortir le caractère de marche ou d'écotone où se trouve la Vallée du Saint-Laurent. Certaines inhibitions à l'accomplissement total du cycle, en effet, auront pour cause immédiate le froid ou l'humidité excessive et les autres, au contraire, la chaleur ou une certaine sécheresse. C'est dire que les premiers dériveront des stades de l'hydrosère et présenteront un caractère plus ou moins nordique et les autres des stades de la xérosère et présenteront un caractère plutôt méridional.

Ces quasiclimax, que l'on désigne parfois (avec les *proclimax* dont il sera question plus loin) sous le nom de climax édaphiques, sont donc déterminés par une certaine lenteur dans l'évolution topographique du site qu'ils occupent, ce ralentissement étant quelquefois assez prononcé pour mettre fin à toute évolution ultérieure, *au cours du présent cycle climatique*, vers l'association climax, vers l'état mésique parfait.

La position relative de ces associations dans les diverses sères de l'aire climatique (*Klimax Gebiet*) est naturellement très importante (Figs 1 et 2), puisque chacune aura retenu des stades précédents un certain nombre de reliques dont la persistance et l'abondance indiqueront à la fois la nature et la gravité des inhibitions subies.

*Il faut se représenter les quasiclimax comme une variation du climax lui-même, c'est-à-dire comme des associations dont les affinités immédiates sont avec le stade terminal plutôt qu'avec un des sous-climax.* La nomenclature que j'emploie servira à le rappeler.

## LES QUASICLIMAX NORDIQUES

Les espèces caractéristiques de tous les quasiclimax sont des mésophytes; nous ne rencontrerons plus désormais d'héliophytes, d'hygrophytes, ni de xérophytes. Il ne saurait non plus être question, dans ces conditions stabilisées, d'espèces non-indigènes.

Les quasiclimax nordiques, tout en présentant les principaux caractères de l'*Aceretum saccharophori laurentianum* décrit plus loin, en diffèrent par la présence d'espèces indicatrices de conditions locales plus froides et souvent plus humides. Ces espèces, en effet, sont caractéristiques et souvent dominantes dans d'autres associations plus boréales appartenant au biome de la forêt canadienne. Quelques-unes d'entre elles sont plus ou moins sur la limite méridionale de leur aire.

## 8. ACERETUM SACCHAROPHORI BETULOSUM (SAF 12, HEIMBURGER VI, VI-O SUBTYPE)

Cette association se trouve le plus souvent dans les ravins froids des régions montueuses et jusque sur des pentes très escarpées (soit 50° environ): ceci entraîne le délavage de l'horizon A et par conséquent l'impossibilité pour cet horizon de se différencier complètement. Tout au plus des paquets ou des flots de sol A sont déposés par les rigoles au pied des arbres. L'exposition est souvent N, NE ou E, par conséquent la lumière et la température y sont relativement basses pour la région. La neige y fond assez tard et souvent le sol demeure humide au printemps et le drainage ne se régularise qu'en été.

L'*Acer saccharophorum* y domine habituellement, tandis que le hêtre en est souvent absent. Le merisier (*Betula lutea*) est l'arbre caractéristique, co-dominant et même parfois dominant. Il y a ordinairement quelques individus d'*Abies balsamea* éparpillés çà et là.

*Faciès à Viburnum-Oxalis.* Il y a deux unions arbustives: l'une à *Viburnum lantanoides* (accompagné parfois de *Corylus cornuta*), l'autre à *Taxus canadensis*. La première est toujours très claire, la seconde peut être extrêmement dense. Les unions herbacées sont très dispersées dans l'ensemble, malgré la haute sociabilité du *Lycopodium lucidulum* et de l'*Oxalis montana*.

*Faciès à Trillium undulatum.* Sur des sols assez fortement acides, en terrain relativement plat, mais où le drainage peut être irrégulier, les caractéristiques sont le *Lonicera canadensis* et à l'union inférieure le *Trillium undulatum*, le *Lycopodium obscurum*, le *Trientalis borealis*.

*Faciès à Dryopteris marginalis.* Sur une forte pente où le délavage de l'horizon A est très prononcé, apparaît le *Dryopteris marginalis*, quelquefois en quantités assez fortes. L'*Hepatica acutiloba* y est souvent très abondant aussi.

*Faciès à Maianthemum-Dryopteris.* Dans les conditions les plus froides, l'*Acer spicatum* forme une union souvent assez dense. Le *Ribes glandulosum* se trouve dispersé parmi l'union herbacée que dominent le *Maianthemum canadense* et le *Dryopteris spinulosa*, auxquels sont associées quelques autres espèces du *Piceetum*, tel le *Clintonia borealis*.

L'érablière du Lac Saint-Jean (à Métabetchouan) appartient à ce dernier type, comme plusieurs de celles de la Gaspésie du reste (Dansereau, 1944 (15)). Or, vers le lac Alex, près du 49° lat. N, l'*Acer saccharophorum* ayant dépassé ses limites, le merisier constitue des peuplements presque purs avec quelques sapins et les synusies frutescente et herbacée décrites plus haut. Une union à *Corylus cornuta* y est particulièrement développée. Cette variation est à rapprocher du type SAF 15 et du *Betuletum abietosum* dont il dérive si souvent.

9. ACERETUM SACCHAROPHORI TSUGOSUM (SAF 10? 11? 17? HEIMBURGER MA-VI, BA-O?)

Cette association ressemble beaucoup à la précédente, et il existe des intermédiaires. Les différences sont probablement dues au drainage, car ce type, très humide au printemps, est susceptible de s'assécher plus que le précédent au cours de l'été. Comme lui, il se rencontre jusque sur des inclinaisons très fortes. C'est donc l'irrégularité du drainage qui en est la condition essentielle. Le pH est inférieur à 7 et peut même être assez fortement acide, comme l'indiquent certaines des espèces herbacées les plus fréquentes.

L'érable à sucre n'est pas toujours dominant. La pruche (*Tsuga canadensis*) est présente, mais très rarement dominante ou subdominante. Les *Fagus grandifolia*, *Acer rubrum* et *Betula lutea* sont présents, ce dernier ordinairement en petit nombre. L'*Acer spicatum* et le *Corylus cornuta* forment une strate frutescente peu fournie, tandis que le *Viburnum lantanoides* et le *Taxus canadensis* sont souvent présents, mais très disséminés.

Diverses unions herbacées se rencontrent ici: l'union à *Osmunda cinnamomea*; l'union à *Trillium undulatum*, *Lycopodium obscurum*, *Trientalis borealis* (où apparaît très fréquemment aussi le *Medeola virginiana*); une autre union à *Mitchella repens*-*Cypripedium acaule*. Dans une autre union, le *Maianthemum canadense* et le *Dryopteris spinulosa* sont tellement abondants qu'il faut bien les mentionner aussi à cause de leur valeur de couverture. Mais, comme le fait remarquer Heimbürger (1941 (33)), ce sont de mauvais indicateurs étant donné leur trop grande plasticité. En fait, ce sont des indices de mésophytie sans plus.

Ce type dérive surtout du *Pinetum Strobi* et il s'apparente dans ses phases les plus jeunes au SAF 10. Quant au SAF 11, il n'existe pratiquement pas dans la Province de Québec. L'un et l'autre sont certainement des sous-climax dans notre région, même si on peut leur accorder le status de climax (Nichols, 1935 (45), Weaver and Clements, 1938 (53)). Quant au type SAF 17, où figure l'épinette rouge (*Picea rubens*), il est très rare aussi.

Il y aurait encore une association *Fagus-Tsuga*, étudiée très en détail par Lutz (1930 (40)), par Hough et Forbes (1943 (35)) et par Odum (1943 (46)), auquel le status de climax a été attribué en Pennsylvanie et dans l'Etat de New York. Ce cas est particulièrement intéressant, parce qu'il fait voir qu'un quasiclimate (et même un sous-climate?) d'une région peut se comporter comme climax dans une autre. Autrement dit, les inhibitions topographiques et microclimatiques qui arrêtent la succession au niveau du quasiclimate peuvent s'étendre à une région entière—et donc à un climat régional

(v.g. les hauts plateaux de la Pennsylvanie)—où elles déterminent *alors* un climax et non plus un quasiclimax.

Je ne crois cependant pas que le *Fageto-tsugetum* de Lutz se présente dans notre région avec la valeur de climax: le *Tsuga canadensis* ne maintient nulle part sa dominance dans notre aire et il ne résiste nulle part à l'invasion de l'*Acer saccharophorum*. Pour les Helderbergs et les Catskills de l'Etat de New York, que j'ai eu l'occasion d'étudier en 1944 à l'*Edmund Nyles Huysck Preserve*, je crois qu'il y a lieu d'émettre à peu près les mêmes doutes. Je me réserve de revenir sur ce point (Dansereau, 1946 (19)).

Quant aux catégories de Heimbürger, le type *Bazzania-Oxalis* figure une phase primitive, tandis que le type *Maianthemum-Viburnum* représente mieux l'association stabilisée.

#### LES QUASICLIMAX MÉRIDIONAUX

Un autre groupe de quasiclimax a une allure et des affinités que l'on peut qualifier de méridionales: les inhibitions de la succession, en effet, favorisent des espèces qui atteignent presque toute leur limite nord-est quelque part dans l'aire de la forêt décidue laurentienne, et même au niveau de l'archipel d'Hochelaga.

Le premier de ces types est plus humide que le climax, tandis que les autres sont généralement plus secs.

##### 10. ACERETUM SACCHAROPHORI ULMOSUM (SAF 13? HEIMBURGER ARIS, DI? MI?)

Dans l'hydrosère de la plaine de débordement, cette association se rencontre toujours et peut durer assez longtemps. On la trouve également sur une toute autre topographie, par exemple sur un flanc de montagne à faible pente (guère plus de 25°) où les eaux d'infiltration maintiennent l'épais humus du sol toujours humide (et non pas seulement frais comme dans les deux types précédents). Le drainage n'est donc pas déficient et il ne s'ensuit aucune stagnation ou congestion, mais la provision d'eau est toujours abondante. La réaction du sol est neutre ou peu s'en faut.

Les arbres forment un dais fermé, de sorte que la lumière est très réduite en été. Les principaux sont l'*Acer saccharophorum* (nettement dominant), le *Fagus grandifolia*, le *Tilia americana* et le *Fraxinus americana*. Il est intéressant de rencontrer l'*Ulmus americana* et le *Juglans cinerea* dans un habitat aussi franchement mésique, car plus au sud, ils ne sortent guère de la plaine de débordement, où se réfugie très souvent aussi le *Tilia americana*. La présence en quantités de ce dernier dans les divers *Aceretum saccharophori* correspond généralement à un manque de densité des arbres. Autrement dit, cette espèce est favorisée, sur un bon sol, par une lumière accrue.

Le *Sambucus pubens* est l'arbuste le plus fréquent. Le *Corylus cornuta* s'y trouve aussi. Le *Dirca palustris*, peu commun dans la Vallée du Saint-Laurent, trouve ici son habitat d'élection.

La strate herbacée ne présente pas au printemps de faciès très différent de celui du climax proprement dit, si ce n'est la présence et souvent l'abondance

très grande de l'*Allium tricoccum*. Les vraies caractéristiques se développent surtout au début de l'été: union à *Adiantum pedatum*, *Laportea canadensis*, *Arisaema atrorubens*; union à *Mitella diphylla*, *Viola canadensis*, *Dryopteris Goldiana*.

Le type 13 de la Society of American Foresters se rapproche de cette association, quoique ce type ne contienne pas de hêtre, comporte un fort pourcentage de tilleul et soit plus ou moins limité au Minnesota, au Wisconsin et au Michigan (où les conditions d'écotone du Saint-Laurent se retrouvent, au moins partiellement).

Le type *Arisaema* de Heimbürger est tout à fait analogue, tandis que ses types *Dicentra* et *Mitella* se rapprochent peut-être davantage du climax vrai.

#### 11. ACERETUM SACCHAROPHORI QUERCOSUM (SAF 51; HEIMBURGER OA)

Au printemps, le sol de ce type est souvent très mouillé, au point d'en être presque marécageux. Au cours de l'été, toutefois, le drainage devient normal et peut même être excessif, entraînant un commencement de sécheresse. En effet le pourcentage de matière organique est souvent faible, et ce site utilise moins bien les précipitations que les autres quasiclimax.

L'espèce caractéristique à l'étage arborescent est le *Quercus borealis*, généralement le var. *maxima*. Dans les cas extrêmes, le chêne rouge est en formation pure. Outre l'*Acer saccharophorum* et de faibles quantités de *Fagus grandifolia*, l'*Acer rubrum* est presque toujours présent, et le *Tilia americana* est assez fréquent, de même que les *Carpinus caroliniana*, *Ostrya virginiana* et *Corylus cornuta*.

L'union herbacée la plus caractéristique est à *Uvularia sessilifolia* (= *Oakesia sessilifolia*), auquel on peut ajouter le *Lycopodium obscurum*, le *Trientalis borealis* et peut-être l'*Hepatica americana*.

Le type "red oak" de l'American Society of Foresters (SAF 52) est tout à fait différent, n'étant guère mésique, mais plutôt un parc qu'une forêt. Peut-être vers sa limite nord, le chêne rouge réagit-il cependant d'une façon analogue à son comportement sur sa limite sud. Il existe une sorte de taillis bas ("scrub") de chênes rouges au Bic.

Par contre, le type SAF 8 en est une forme primitive, dérivant évidemment du *Pinetum Strobi*. Il en va de même du type *Aralia-Oryzopsis* de Heimbürger qui tend vers son type *Oakesia*, beaucoup plus mésique.

C'est sans doute dans la Vallée de l'Ottawa que cette association est le plus développée, et particulièrement du côté ontarien où les effets de la sécheresse sont évidents. Dans le reste de l'aire, l'*Aceretum saccharophori quercosum* est très faiblement représenté.

#### 12. ACERETUM SACCHAROPHORI CARYOSUM (SAF 57; HEIMBURGER M1)

Cette association et la suivante sont, à vrai dire, beaucoup plus proches du climax que toutes les précédentes sauf l'*Aceretum saccharophori ulmosum*. L'influence modifiante ici, par rapport au climax, est le réchauffement précoce

au début de la saison. L'exposition donc sera généralement sud ou sud-ouest et plus ou moins à l'abri des vents froids, sinon des vents desséchants. Le sol aura accompli une évolution très poussée, mais la couche d'humus pourra être moins épaisse que dans le climax, assurément moins que dans le numéro 10. Le sol sera aussi légèrement acide.

L'érable à sucre et le hêtre sont toujours dominants. Cependant quelques espèces caractéristiques d'un climax voisin (*Quercetum albae*) y figurent: *Carya cordiformis*, *C. ovata*, et quelquefois *Prunus serotina*, *Quercus alba*, *Q. macrocarpa* et *Hamamelis virginiana*. Les arbustes sont ceux du climax, de même que la plupart des espèces herbacées. Toutefois, quelques espèces se distinguent: *Parthenocissus quinquefolia* et *Smilax herbacea*. Les *Trillium grandiflorum*, *Uvularia grandiflora* et *Hydrophyllum virginianum* sont à leur optimum ici. La plupart de ces espèces ont leur maximum d'expansion à l'ouest des Appalaches, sous un climat où la sécheresse va en augmentant.

Ce type ne dépasse pas considérablement l'archipel d'Hochelaga: la moindre altitude, dans les Cantons de l'est et les Laurentides, fait obstacle à son expansion.

### 13. ACERETUM SACCHAROPHORI NIGROIDES

Ce quasiclimax est encore plus rare et plus limité dans notre aire. Il est assez difficile de savoir s'il trouve son équivalent ailleurs, la littérature forestière n'étant guère prodigue d'informations au sujet de l'érable noir (*Acer nigrum*) que l'on confond volontiers avec l'érable à sucre. Il se produit d'ailleurs entre les deux espèces de nombreuses hybridations et recombinaisons (Marie-Victorin, 1935 (42), Dansereau et Lafond, 1941 (21), Dansereau et Desmarais, 1946 (20)).

L'*Aceretum saccharophori nigroides* diffère du climax et du type précédent surtout par la présence de l'érable noir dans l'union arborescente. Les unions arbustive et herbacée sont la plupart de celles des numéros 12 et 14.

On peut encore rapprocher ce type de l'*Aceretum saccharophori ulmosum*, car il arrive que le sol riche ait une haute capacité de rétention de l'eau. Plusieurs auteurs (dont Deam, 1940 (22)) attribuent à l'érable noir un habitat plus humide que celui de l'érable à sucre. Dans notre région, il ne se rencontre strictement que sur un substratum calcaire, même si ce substratum est dolomitique. Il y a lieu de croire que ses limitations sont d'ordre thermique plutôt que chimique. En tout, seules les stations suivantes sont connues: Saraguay, Cartierville, et Sainte-Anne-de-Bellevue (Ile de Montréal); Saint-François-de-Sales et Laval-des-Rapides (Ile Jésus); Saint-Eustache (comté des Deux-Montagnes); Grenville (comté d'Argenteuil); Saint-Lin (comté de l'Assomption); Saint-Armand (comté de Missisquoi).

## Le climax

### 14. ACERETUM SACCHAROPHORI LAURENTIANUM

L'érablière laurentienne atteint son climax total sur des sites bien drainés, dont la pente n'excède guère 35°, et où l'évolution du sol a atteint son terme par la différenciation maximum des horizons. Les facteurs de déséquilibre

auront disparu: froid ou chaleur excessifs, humidité et sécheresse, bref tout ce qui est contraire à l'état mésique. Il a été démontré par beaucoup d'auteurs que les facteurs météorologiques affectent une allure à la fois régulière et modérée dans les climax. C'est dire que les variations tant quotidiennes que saisonnières de la température et de l'humidité s'inscrivent entre des extrêmes plus rapprochés que ceux des autres habitats environnants, et s'expriment par des courbes plus aplaties.

Ceci, naturellement, est dû à l'effet-tampon d'une strate arborescente dense qui isole le milieu sylvestre de l'atmosphère ambiante et dont la stabilité floristique est l'expression même de cette adaptation finale au climat régional que figure toujours le climax. Il n'est peut-être pas superflu d'insister de nouveau sur le fait que cette association est celle qui utilise au maximum les ressources du milieu. Elle est aussi la seule dont les espèces, tout en ayant des exigences primaires communes, ne manifestent pratiquement plus de compétition active, parce qu'elles ont en même temps des exigences secondaires complémentaires qui leur permettent d'occuper chacune un biotope particulier et de réaliser un parfait commensalisme.

Dans l'union macrophanérophYTE, l'arbre dominant est l'*Acer saccharophorum*, qui dans certains cas se rencontre en formations pures (la 'consociation' de Clements). Le *Fagus grandifolia* lui est généralement associé quoiqu'il n'atteigne à peu près jamais une proportion de plus de 20%. Les *Fraxinus americana* et *Tilia americana* sont fréquents aussi, mais toujours en nombre réduit. Quelques petits arbres (union mésophanérophYTE), qui ne percent pas la voûte feuillée, sont très caractéristiques: l'*Acer pennsylvanicum* et l'*Ostrya virginiana*. Les principaux arbustes sont le *Cornus alternifolia* et le *Sambucus pubens* (union microphanérophYTE).

Il n'y a donc, aux synusies supérieures, aucune espèce à feuilles persistantes. Par conséquent le facteur lumière va manifester une telle variation que les aspects saisonniers seront très différenciés, et les caractéristiques vernales, estivales, sérotinales et automnales très différentes quant aux formes biologiques et à l'abondance.

Les conditions printanières, avec leur éclaircissement maximum (75% dans le *Fagetum sylvaticae* de Tchécoslovaquie (Braun-Blanquet, 1932 (3)), vicariant biogéographique de notre *Aceretum saccharophori*) favorisent les géophytes. Ces espèces ont un cycle vital généralement très court et même ne laissent aucune trace dans la forêt quand l'ombre s'y est étendue: le *Claytonia caroliniana*, le *Dicentra Cucullaria*, le *Dicentra canadensis*, l'*Erythronium americanum* durent quelques semaines seulement, produisent feuilles, fleurs et fruits, et reconstituent les réserves de leurs parties souterraines. D'autres débutent très tôt, produisant leurs fleurs puis leurs feuilles (*Hepatica acutiloba*, *Carex pedunculata*, *Carex plantaginea*), ou leurs fleurs et leurs feuilles (*Trillium erectum*, *Sanguinaria canadensis*, *Viola eriocarpa*, *Polygonatum pubescens*, *Smilacina racemosa*, *Asarum canadense*, *Caulophyllum thalictroides*) et continuant leur végétation et le mûrissement de leurs fruits dans l'ombre grandissante. Toutefois, en juillet et août le nombre des individus portant encore des feuilles



et des fruits est généralement faible en comparaison du nombre qui a fleuri. La couverture totale de ces espèces vernales, la plupart très grégaires, peut être très élevée, au point de former un tapis quasi-continu ou bien consister en touffes isolées au pied des arbres et autour des pierres.

La période estivale, qui marque leur disparition ou en tout cas leur extrême réduction, présente un aspect très différent. Plusieurs espèces, apparues de bonne heure, ont fleuri ou fructifié et fait une certaine croissance pendant la période vernale, mais ne se développent pleinement qu'en été: les *Dryopteris spinulosa*, *Polystichum acrostichoides*, *Osmorhiza Claytoni*, *Actaea rubra*, *A. alba* et *A. pachypoda*. Quelques hémicryptophytes produisent une rosette ou quelques feuilles au printemps, puis se développent et fleurissent en juin et juillet, tels les *Carex arctata*, *Prenanthes altissima*, *P. alba*, *Sanicula trifoliata*, *S. marilandica*. D'autres n'apparaissent guère avant les chaleurs, tels les *Desmodium grandiflorum*, *Aralia racemosa*, *Amphicarpaea bracteata*, *Phryma leptostachya*.

La période sérotinale est caractérisée par la persistance en feuilles ou en fruits d'un certain nombre d'espèces vernales et surtout estivales et par la floraison de quelques espèces brévidiurnes—dont les premières feuilles ont pu apparaître en mai—tel les *Solidago latifolia*, *Aster acuminatus* et *Eupatorium urticaefolium*. Cette saison est celle de la maturation des fruits et des graines. Elle coïncide assez souvent avec une relative sécheresse de l'atmosphère et un abaissement de la nappe phréatique. A vrai dire ni l'un ni l'autre de ces phénomènes ne se fait beaucoup sentir dans l'*Aceretum saccharophori laurentianum*, du moins au niveau de la synusie herbacée, car le sol superficiel ne perd pas sensiblement de son eau.

Aussi la période automnale qui suit n'est-elle pas marquée par des changements brusques, malgré le commencement de la chute des feuilles, l'abaissement de la température, le retour des pluies et la hausse de la nappe phréatique. Il est très remarquable, en effet, si l'on compare la végétation herbacée du climax avec celle d'un *Pinetum Strobi* ou d'un *Betuletum* avoisinant, que les feuilles sont atteintes moins tôt par la gelée, toujours en vertu de l'effet-tampon du climax sur le climat régional (Hough, 1945 (34)). On voit même assez fréquemment, pendant l'été de la Saint-Martin ("Indian Summer") le *Viola canadensis* fleurir de nouveau, tandis que l'*Osmorhiza Claytoni*, le *Galium triflorum* et d'autres espèces, à la base de leurs tiges plus ou moins sèches, produisent des feuilles nouvelles qui demeureront vertes sous la neige.

Le climax est une association bien équilibrée; c'est la seule qui soit contrôlée par le climat et non par le sol (Clements, 1936 (11)). Elle est sujette d'autre part à ce que Weaver et Clements (1938 (53)) ont appelé l'*annuation*, c'est-à-dire à des modifications plus ou moins graves, d'une année à l'autre ou au cours de périodes (cycliques ?) d'années, des proportions relatives des espèces présentes aux diverses synusies. Ceci peut même affecter la dominance. Ainsi, plusieurs années sèches de suite peuvent diminuer le nombre des *Trillium erectum*, *Viola eriocarpa* dont les rhizomes sont peu profonds; les années

humides favoriseront l'*Athyrium angustum*, l'*Adiantum pedatum*, le *Dicentra Cucullaria* dont les feuilles sont fragiles, etc.

D'autres conditions locales sont susceptibles de modifier quelque peu le faciès du climax, au moins sur de faibles étendues. Ainsi le chablis, la chute de vieux et grands arbres, phénomènes qu'il faut bien considérer comme faisant partie du cycle naturel, ont pour effet de créer des puits de lumière qui favoriseront le *Tilia americana*, le *Prunus serotina* (SAF 14-A, Hough and Forbes, 1943 (35)) et même le *Pinus Strobus*. C'est en considération de ce facteur—à l'échelle de centaines de milles carrés—que Nichols (1935 (45)) et Whitford (1901 (55)) reconnaissaient au pin blanc une place dans le climax.

Le vent, surtout dans les parties marginales de la forêt, ou en présence de sols peu profonds, favorise probablement la régénération du *Fagus grandifolia* plutôt que de l'*Acer saccharophorum*, et permet même la formation de petites "consociations" locales. La partie haute de l'Ile d'Orléans, très exposée aux vents du nord-est et du sud-ouest, porte de véritables *Aceretum-Fagetum*, peut-être en réponse à l'augmentation de l'évaporation.

Tel est donc le thème central de l'érablière laurentienne autour duquel s'ordonnent quelques variations mineures qui viennent d'être décrites, et aussi des variations plus grandes appelées quasiclimax.

Grâce à un tableau d'association, basé sur plusieurs centaines de relevés dans l'*Aceretum saccharophori* faits depuis 1940, il sera possible d'appuyer les considérations précédentes sur des chiffres d'abondance, de sociabilité, de fréquence, de présence et de constance (voir Braun-Blanquet, 1932 (3)), d'estimer la fidélité et de faire usage des valeurs d'indice déjà proposées (Dansereau, 1943 (14)). Cette description quantitative de la forêt décidue laurentienne permettra de la comparer à ses équivalents dans le nord et l'est des Etats-Unis autrement que sur une base purement floristique (voir Dansereau 1943 (14), Tableau I). De même, les aspects saisonniers (l'*aspection* de Clements) feront l'objet d'une étude détaillée.

Je crois cependant en avoir donné pour le moment une description suffisante pour faire ressortir l'originalité du complexe phytosociologique qu'est l'*Aceretum saccharophori laurentianum*. Ce climax de la forêt décidue du Saint-Laurent est le leitmotiv autour duquel s'ordonnent, selon les fluctuations de quelques facteurs physiques, des variantes de relativement peu d'ampleur nommées ici quasiclimax.

Il ressort également que l'*Aceretum saccharophori* ne correspond exactement à aucun des types de la Society of American Foresters quoiqu'il se rapproche des numéros 14 et 57. Il correspond d'autre part assez bien aux types *Dicentra* et *Mitella* de Heimbürger.

### Les disclimax

Tout ce qui précède a trait à la *prisère*, c'est-à-dire aux diverses successions qui convergent vers un même climax dans les conditions naturelles. L'intervention plus ou moins délibérée et fréquente de l'homme introduit des per-

turbations plus ou moins graves qui nous obligent à superposer, sur le motif ("pattern") de la Fig. 1, des variations nouvelles: c'est la *subsère*.

Les interventions humaines sont de plusieurs sortes: coupe, éclaircissage, feu, pâturage, drainage, inondation. Il est admis que ces interventions ont pour effet de produire une régression, c'est-à-dire une marche-arrière dans la succession. Plus exactement, on se représente une sorte de récapitulation: un climax est détruit, et la succession recommence, soit à partir du sous-climax si l'intervention est bénigne, soit à partir d'un stade pionnier si elle est plus grave (voir Fig. 7). Or, ceci ne se vérifie que si l'intervention en question atteint également les diverses synusies de la végétation et les divers horizons du sol, ce qui est non seulement improbable mais rare. Pour revenir aux considérations exposées plus haut au sujet de l'évolution différentielle des synusies et des horizons, l'intervention ne pourra causer une récapitulation de la succession primaire que si elle s'exerce non seulement à tous les niveaux mais à tous les niveaux à la fois pour cesser immédiatement après.

Or, dans la pratique, les interventions directes, et surtout indirectes, de l'homme sont progressives ou irrégulières. Une intervention continue et bénigne est même susceptible de causer une véritable rétrogression, c'est-à-dire une succession à l'envers. Frankton et Raymond (1944 (26)) nous font voir, dans notre région, les rétrogressions: *Trifolietum* → *Festucetum* → *Danthonietum* et *Trifolietum* → *Agrostidetum* → *Festucetum*, dues à un facteur biotique continu, le pâturage. La suppression de ce facteur induirait en peu de temps la réversion à la succession (progressive) normale: *Danthonietum* → *Festucetum* → *Trifolietum*, etc. (voir Figs 1 et 2).

Or, si les interventions de l'homme sont susceptibles, en altérant les conditions physiques et biologiques du milieu, d'induire de simples récapitulations et rétrogressions, elles produiront nécessairement aussi des modifications favorables à la constitution d'associations qui n'existent nulle part dans la nature à l'état spontané. Tout au moins peut-on se représenter les conditions créées par l'homme, tant dans la conjonction des facteurs que dans la durée qu'elles affectent, comme particulièrement favorables à des espèces qui ne dominent dans aucun habitat "naturel", dans aucune des diverses niches qui se constituent au cours de l'évolution topographique régionale, sous le climat actuel. Il y a longtemps qu'on a reconnu ce principe à propos des mauvaises herbes, qui vivent ici, en pays froid et humide, dans une micro-steppe sans équivalent naturel régional, à preuve qu'on n'y rencontre peu ou pas d'espèces indigènes.

Par conséquent, si l'intervention de l'homme, dans le climax de la région qui nous intéresse, a pour effet de créer des conditions analogues à celles des quasyclimax, ce sont ces dernières associations qui occuperont des sites légèrement dégradés. Or, la plupart du temps, la hache et le feu surtout, auront des effets beaucoup plus graves et ravalent le site au moins au niveau d'un sous-climax et même d'un stade pionnier. Tandis que des interventions plus bénignes créeront des conditions tout à fait nouvelles auxquelles les plantes

répondront en formant des faciès particuliers et caractéristiques. La répétition périodique de ces interventions conservera à ces faciès une permanence dans le temps qui justifie leur désignation de disclimax.

#### LES DISCLIMAX BUCHÉS

La coupe sélective et l'abattage des arbres se rencontrent à tous les degrés, depuis l'éclaircissage qui ne fait qu'accélérer les derniers stades de la succession jusqu'à la coupe à blanc estoc (Fig. 7). Il ne sera question pour le moment que des coupes relativement bénignes, puisque cette étude a pour objet les associations arborescentes voisines du climax et non les sous-climax, et encore moins les stades pionniers.

La coupe sélective, dans la province de Québec, a pour objet de favoriser le remplacement du hêtre, du noyer, de la pruche, du frêne, du chêne rouge, du merisier, du tilleul ou de l'orme par une population pure d'érable à sucre (voir SAF 14) pour fins d'exploitation du sirop et du sucre.

Pour des raisons connexes—faciliter la circulation des chevaux dans l'érablière, par exemple—on détruit très souvent la strate frutescente tout entière, abattant les jeunes *Acer saccharophorum* en même temps que les *Cornus*, *Sambucus*, *Corylus*, etc.

Enfin, on "nettoie" l'érablière, en enlevant tout le bois mort, toutes les branches qui tombent, les arbres renversés, etc. Considéré au simple point de vue écologique, le nettoyage est une intervention peut-être aussi importante que la coupe. Les arbres morts—et même les arbustes et les branches—jouent un certain rôle dans la dynamique du sol, dans le renouvellement de l'horizon A qui dépend de la décomposition des matières organiques. Certes, ce facteur est plus important dans les forêts de conifères que dans les bois décidus, car dans un *Piceetum*, nombre de plantes herbacées et les plantules de l'épinette elle-même ne peuvent avoir prise que sur le sol minéral (isolé par un tapis de mousses, un écran d'aiguillettes, ou un humus stagnant) ou sur un tronc renversé en décomposition. Le rajeunissement de l'horizon A cependant, dépend aussi dans l'érablière des décompositions ligneuses dans une certaine mesure. Il est très remarquable, par exemple, que le merisier germe très souvent sur les souches mortes des érables, du hêtre ou de la pruche.

La coupe, donc, aura des répercussions diverses selon sa gravité, sa fréquence et l'association sur laquelle elle aura été pratiquée: le climax ou l'un quelconque des quasiclimax. Ses effets seront généralement de l'un des trois types décrits ci-après, lorsque la voûte de la strate arborescente n'aura pas été trop fortement diminuée, mais lorsque la strate arbustive aura été à peu près détruite.

#### 15. ACERETUM SACCHAROPHORI DENNSTAEDTIOSUM

Ce disclimax se formera surtout à la suite d'une coupe sélective soit dans le climax (no 14) soit dans une érablière humide et plutôt froide (nos 8 et 9), plus rarement après une intervention en milieu plus sec (nos 12 et 13). Ici

la densité de la strate arborescente est souvent réduite, et d'ailleurs la population est fréquemment jeune. Il est probable que ce faciès ne se forme pas très spontanément mais à la suite d'interventions habituelles au cours de très longues périodes.

La densité plutôt réduite des arbres et l'absence totale d'arbustes permettent au soleil de faire fondre très rapidement la neige. La flore printanière ne diffère pas sensiblement de celle du climax. Ce n'est guère qu'à la phase estivale que la dégradation devient apparente. A ce moment, en effet, une fougère, le *Dennstaedtia punctilobula*, surgit: elle est souvent assez abondante pour couvrir d'une formation pure le plancher du bois. Cette espèce est peu exigeante et manifeste une adaptabilité assez grande, puisqu'elle est à la fois euryphotique, euryhygrique et eurytherme.

On ne peut s'empêcher de constater au sujet de cette fougère les possibilités d'évolution considérablement accrues que lui vaut cet habitat semi-naturel. En effet, sa place dans la prisère est très restreinte. On ne la trouve aujourd'hui à peu près jamais dans un habitat intact: elle devait être limitée à l'orée des bois autrefois, et ne formait certainement pas les vastes colonies pures qu'elle constitue aujourd'hui. L'étude génétique des populations a bien fait ressortir l'importance des nombres d'individus dans une aire donnée. Il me semble que les grandes synthèses récentes sur la question de l'évolution et de la sélection naturelle n'ont toutefois pas suffisamment mis en lumière l'importance du climax et des successions dans la phylogénie des groupes végétaux et animaux. Il serait intéressant de comparer le degré de stabilité génétique d'un grand nombre d'espèces avec leur status dans la succession: les espèces du climax (milieu stabilisé) sont dans l'ensemble moins grégaires, ont une sociabilité réduite, tandis que celles des sous-climax et surtout des associations pionnières (milieux instables) sont très souvent grégaires. Ainsi, comparons le *Maianthemum canadense* au *Trillium undulatum* ou le *Solidago canadensis* au *Solidago latifolia*: la variabilité est plus élevée chez le *Maianthemum* et le *S. canadensis*, qui apparaissent plus tôt dans la sère que les deux autres espèces.

Le cas du *Dennstaedtia punctilobula* nous rappelle aussi la théorie si féconde émise par Griggs (1940 (30)): les plantes rares se rencontrent dans des habitats pionniers. Beaucoup de ces plantes rares sont des endémiques, telles les épibiotés du Bas Saint-Laurent. D'autres sont aujourd'hui des "mauvaises herbes", telle l'herbe-à-poux (*Ambrosia artemisiifolia*), tenues en échec par l'équilibre naturel et libérées par l'homme. La dennstaedtie (la plus proche parente dans notre flore des fougères arborescentes tropicales) doit donc son apparente agressivité au maintien de conditions "artificielles": sa valeur de compétition n'est élevée qu'à cause de sa capacité d'utiliser la conjonction de facteurs d'un habitat jamais réalisé par la nature sous le climat qui lui permet de survivre.

L'*Aceretum saccharophori dennstaedtioides* abritera aussi quelques *Prunus virginiana*, *Rubus idaeus*, *R. occidentalis* et *R. allegheniensis*. En été, un certain nombre de mauvaises herbes apparaitront dans les endroits où la

couverture arborescente est la moins dense: *Prunella vulgaris*, *Hieracium* spp., *Taraxacum officinale*, *Antennaria* spp., etc.

#### 16. ACERETUM SACCHAROPHORI GLYCERIOSUM

Ce faciès ne pourra se former que sur un substratum déjà humide, soit à partir des types 8, 9 et 10. La suppression (pas nécessairement sélective) des arbres causera ici une diminution du pouvoir d'évaporation de la forêt et une hausse de la nappe phréatique. Il y aura donc un commencement de paludification. Le drainage sera congestionné et les espèces qui réclament une bonne aération du sol seront éliminées. Le pH du sol (plutôt élevé dans le type 10) tombera jusqu'à 6 environ et même beaucoup plus bas. Un tel milieu ne se réchauffe que très lentement au printemps, et il demeure même inondé par endroits assez longtemps, au point que quelques petites flaques d'eau peuvent persister jusqu'en été. En tout temps, c'est un site plutôt hétérogène, car de petites différences de relief (8-15 pouces) créent des biotopes favorables à des espèces fort différentes. Il se forme surtout de petites buttes arrondies de consistance plus dense mais un peu mieux aérées que le sol sous-jacent. Comme l'a signalé Romanovsky (1943 (47)), ces formations ont pour origine des différences de température et l'action du gel. Elles sont très communes dans les régions froides (sols 'polygonaux') mais se rencontrent aussi dans la zone tempérée. Dans les érablières pâturées, le piétinement contribue pour une grande part à maintenir ces conditions en modifiant la structure du sol.

La flore vernale perd toutes les espèces sensibles à l'aération du sol, au pH bas, à l'humidité excessive: *Dicentra Cucullaria*, *Hepatica acutiloba*, *Mitella diphylla*, et même jusqu'à un certain point *Erythronium americanum*, *Trillium erectum*, *Allium tricoccum*.

L'union herbacée se répartit sur une mosaïque de biotopes dont chacun occupe des proportions variables.

*Biotope à Dryopteris noveboracensis*, sur les parties les plus hautes des petits tertres mentionnés plus haut, où le sol s'assèche considérablement en été. On retrouve là le *Dennstaedtia punctilobula* et l'*Hieracium vulgatum*.

*Biotope à Maianthemum*, sur les flancs de ces petits tertres ou tout au moins sur les parties de sol toujours frais mais libéré dès le printemps de son excès d'eau. Avec le *Maianthemum canadense*, d'autres espèces nordiques et acidophiles: *Clintonia borealis*, *Dryopteris spinulosa*, *Viola pallens*.

*Biotope à Coptis*. Les parties plus humides du flanc de ces tertres seront couvertes de mousses (*Dicranum undulatum*, *Calliergonella Schreberi*, *Polypodium* spp.) et de *Coptis groenlandica*.

*Biotope à Glyceria*. Les dépressions, entre les tertres, endroits de drainage difficile, seront colonisées par de véritables hygrophiles: *Glyceria striata*, *Onoclea sensibilis*, *Carex intumescens*, *Impatiens biflora*.

La reproduction des arbres est plutôt lente: l'*Acer rubrum* et le *Betula lutea* sont favorisés, germant assez bien dans le biotope à *Maianthemum*. De telle

sorte qu'il peut se reconstituer dans ces conditions un *Aceretum rubri* ou même un *Betuletum abietosum* avant que le quasiclimax ou le climax même ne recouvrent le site.

#### 17. ACERETUM SACCHAROPHORI ACEROSUM

Ce type, très commun dans toutes les régions où l'érable est exploité pour le sucre, dérive le plus souvent du climax (no 14), ou encore des quasiclimax nos 10, 12 et 13. Il s'agit donc de peuplements ayant subi une coupe répétée et continue, et qui a pu être sélective au début (voir type SAF no 14). Souvent, le diamètre moyen des arbres est assez faible (par exemple 8-10" d.h.p.) et les érables encore jeunes. La note la plus caractéristique, ici encore comme dans les deux types précédents, est l'absence d'une strate arbustive bien définie et la très grande abondance de la régénération de l'*Acer saccharophorum*.

Dans les conditions équilibrées du climax, la germination et la croissance de l'espèce dominante sont excellentes; aussi y rencontre-t-on des individus de tous les âges et diamètres répartis d'une façon plus ou moins insensible. Une régénération\* intense ou la présence dans une synusie quelconque d'un très grand nombre d'individus d'une même espèce sont généralement un signe d'instabilité et souvent d'intervention. En effet, une forte densité d'une mégaphanérophite à une synusie inférieure ne peut résulter que du soulagement d'une pression écologique. L'écèse réalisée chaque année ou périodiquement par les plantules de l'*Acer saccharophorum* est une réponse au vide écologique créé par la suppression des arbustes. Cette invasion, et surtout la croissance touffue qui s'ensuivra au cours des quelques années suivantes, sont certainement favorisées par une lumière plus grande que celle du climax. En effet, parmi les espèces sylvestres arborescentes, c'est surtout pendant la période critique de la germination que la plante est sciophile, car elle fait une meilleure croissance en pleine lumière, comme Watt (1925 (52)) l'a démontré pour le *Fagus sylvatica*, le vicariant écologique européen de notre *Acer saccharophorum*.

Un autre facteur est l'humidité du sol. Les deux types qui précèdent sont, l'un trop sec et trop fermé au niveau herbacé (no 15), et l'autre trop humide (no 16), et favorisent d'autres espèces que l'érable à sucre. La graine de l'érable à sucre est très sensible—plus que celle des autres érables—à la dessiccation (Jones, 1920 (37)), et il lui faut, de plus, une période de repos à basse température (Stevens and Dunn, 1943 (50)). D'autre part, la graine semble être produite en plus grandes quantités dans des formations plus ou moins intactes. Baldwin (1942 (2)) donne des chiffres qui varient du simple au double pour un bois où l'on a pratiqué une (forte ?) coupe sélective, et pour une forêt vierge, dans le Michigan.

\* Ce mot (Westveld, 1939 (54)) est employé ici de préférence à reproduction, une expression courante en sylviculture. Au point de vue strictement biologique, la reproduction ne concerne que la fécondation et la germination. Les forestiers toutefois en étendent l'usage à toute la phase critique qui va de la fécondation jusqu'aux quelques premières années de croissance. Ils y incluent aussi la propagation végétative, et parlent de la plantation comme d'une "reproduction artificielle" (Toumey and Korstian, 1937 (51)).

Pour que ce type se réalise, il faut donc: une augmentation de la lumière, le maintien de bonnes qualités dans le sol et une provision suffisante de graines. Ces diverses conditions ne se rencontreront que si la dégradation n'est pas trop prononcée et surtout n'affecte presque pas l'horizon A du sol. La lumière accrue—par exemple sur un site *Aceretum saccharophori ulmosum*—affaiblit la valeur de compétition des espèces herbacées estivales et favorise les plantules d'érable à sucre. La bonne qualité du sol lui-même ne permet guère à des espèces arborescentes non-mésiques d'y germer ou en tout cas de s'y développer en résistant à la compétition.

D'autre part, la poussée très dense de ces plantules étouffe toute autre forme de végétation à la strate herbacée, sauf les espèces les plus printanières. C'est même sur un tel site que l'*Erythronium americanum*, le *Trillium grandiflorum*, le *Claytonia caroliniana* forment les populations les plus considérables. L'érythronium mûrira de plus grandes quantités de fruits. La survivance des espèces estivales, d'autre part, est beaucoup plus compromise par la compétition des jeunes érables, et leur nombre diminue sensiblement. Cette flore se reconstituera peu à peu, à mesure que le choc causé par une forte compétition unistrate se détendra par une croissance de plus en plus répartie sur plusieurs synusies. Or, ce même choc se répétera chaque fois qu'on supprimera la strate herbacée et, si cette pratique est répétée périodiquement, la régénération de la strate herbacée par les éléments originaux sera de moins en moins facile.

#### LES DISCLIMAX PÂTURÉS

Les animaux domestiques, et surtout les bovins, sont admis dans un grand nombre d'érablières en été. Il ne s'agit pas de discuter ici les avantages très contestables qui en résultent pour l'industrie laitière, mais plutôt de constater l'effet de ce facteur biotique sur la dynamique forestière.

Dans un certain nombre de cas, l'addition du pacage aux dégradations causées par la hache n'est pas considérable, soit que le nombre de bêtes soit très réduit ou que la dégradation déjà encourue les éloigne plus ou moins définitivement. Ces cas se trouvent déjà prévus sous les types précédents.

Il s'agit plutôt de stations, ordinairement peu étendues, où l'influence des animaux est prépondérante, soit à cause de leur nombre, soit à cause d'une vulnérabilité particulière de la forêt due à des interventions antérieures.

L'action du bétail a les effets suivants: le piétinement détruit les racines superficielles des arbres ou, en les blessant, les expose à des infections; le broutage, le frottement et l'arrachage des jeunes plants empêchent la régénération ou en changent la composition botanique (Lutz, 1930 (41)). De plus, le tassement du sol en altère la structure et, par conséquent, l'humidité, l'aération (Chandler, 1940 (9)) et le drainage, et peut entraîner une chute du pH, toutes conditions éliminatoires pour beaucoup de plantes herbacées et pour les plantules de certains arbres. Il faut ajouter que la suppression de la synusie frutescente permet parfois au vent de pénétrer dans la forêt et que les branches des arbres sont ainsi brisées, sans compter son effet desséchant sur la végétation herbacée et sur l'horizon A du sol.



La disparition des arbustes favorise une écèse assez soudaine et forte dans la synusie herbacée de l'*Aceretum saccharophori acerosum*. Si le pâturage s'ajoute à cette perturbation, les résultats pourront être de plusieurs sortes, selon le point de départ, et surtout la nature du sol original.

#### 18. ACERETUM SACCHAROPHORI PASCUORUM

Il est d'autant plus difficile de décrire adéquatement ce type qu'il est extrêmement hétérogène. Une même station présentera une mosaïque de biotopes secs et humides, ombragés et éclairés. Un relevé complet de la végétation herbacée, dans un cas semblable, sera très intéressant, car si l'on accorde une valeur d'indice aux espèces (Dansereau, 1943 (14)) et qu'on multiplie cette valeur par leur abondance, on pourra estimer le sens de la dégradation et l'état actuel de l'équilibre écologique. Ainsi dans les deux relevés que nous donne Lutz (1930 (41)) pour des forêts décidues vierges et pacagées, la constitution de plusieurs biotopes saute aux yeux, puisque quelques espèces n'ont à peu près pas été dérangées tandis que la disparition ou l'augmentation des autres indiquent à la fois une augmentation de l'intensité lumineuse et des modifications locales du sol ici plus sec et là plus humide.

Dans la région laurentienne, l'effet du changement de signe des facteurs écologiques principaux peut se définir ainsi qu'il est indiqué au Tableau IV. Plusieurs espèces sont sensibles à plus d'un facteur, bien entendu, et surtout les espèces très spécialisées du climax. Ce tableau prétend seulement donner une idée de leur *réponse immédiate* à une faible variation de lumière, chaleur, humidité ou pH.

*Espèces du climax.* Certaines espèces du climax (Dansereau, 1943 (14), Tableau III, indice 5 (SME) pour la plupart) sont d'une grande sensibilité et disparaissent généralement dès la première atteinte à l'équilibre primitif (*Asarum canadense*, *Dicentra Cucullaria*, *Trillium erectum*); d'autres manifestent une assez forte résistance (*Carex arctata*, *Trillium grandiflorum*); et d'autres enfin augmentent (*Maianthemum canadense*, *Dryopteris spinulosa*, *Trillium undulatum*). On constatera généralement que ces dernières ont leur optimum dans un sous-climax ou un quasiclimax et par conséquent trouvent, dans une rétrogression, des conditions favorables (au moins à la propagation végétative, sinon à la reproduction). Il est bon d'insister sur ce point, car tous les cas de persistance sont loin d'être équivalents, puisqu'ils sont attribuables à des facteurs différents. Ainsi l'*Erythronium americanum* se trouve très bien pendant de nombreuses années d'une exposition à la pleine lumière. Il arrive même qu'il fructifie davantage que sous bois. Seule la dégradation assez avancée du sol (à moins que ce soit l'action brutale de la charrue) l'éliminera, car il peut apparemment s'ajuster à des fluctuations du niveau de l'eau (Dansereau, 1945 (17)). D'autre part, l'accroissement immédiat du *Dryopteris spinulosa* et du *Maianthemum canadense* sera tôt suivi d'une décroissance si la lumière demeure très forte.

*Espèces des quasiclimax et sous-climax* (Dansereau, 1943 (14), Tableau IV à VI). L'écologie particulière et la position dynamique de chacune de ces

associations ont été définies plus haut assez en détail pour que je n'aie pas à y revenir. La conjonction de facteurs responsable de chacun de ces équilibres ou de ces stades dans l'évolution de la végétation toutefois donne successivement la prépondérance à la lumière, à la chaleur, à l'humidité et à la réaction du sol.

Les dommages causés au climax ou à l'un quelconque des quasiclimax par la paissance se traduiront donc, ainsi qu'il est indiqué au Tableau IV, par des changements dans le status phytosociologique de diverses espèces. Le groupe de celles qui se montrent tolérantes vis-à-vis d'un drainage irrégulier—et qui sont bien représentées dans l'*Aceretum saccharophori tsugosum*—aura d'excellentes chances de succès dans une érablière pâturée où la strate arborescente n'est pas encore entamée. Tandis que les hygrophiles—peut-être déjà présentes dans les rares dépressions humides de la forêt primitive—verront s'agrandir le biotope qui leur est favorable.

L'augmentation considérable de l'*Oxalis montana* remarquée par Lutz (1930 (41)) s'explique sans doute d'abord par la chute du pH et par la résistance de cette espèce à un drainage moins régulier.

*Espèces pionnières* (Dansereau, 1943 (14), Tableaux VII à IX). La plupart de ces espèces réagissent très vivement à la lumière surtout; bon nombre d'entre elles sont même d'origine méditerranéenne et ne se rencontrent nulle part en Amérique du Nord-Est dans des habitats "naturels".

Assurément, ces espèces, dans certaines érablières dégradées, constituent presque à elles seules le tapis herbacé. Lutz (1930 (41)) signale l'augmentation très notable de toute la synusie herbacée après la pâture. Il ajoute que "over most of the area the humus conditions appear considerably better than on the ungrazed area. Rather rapid nitrification is indicated by the fairly common occurrence of species which are generally regarded as nitrophilous". Il est à remarquer que parmi ces nitrophiles, celles qui réussissent le mieux (à en juger par leur fréquence, car Lutz ne donne pas de chiffres pour l'abondance) sont également héliophiles: *Plantago major*, *Stellaria media*, *Polygonum Hydropiper*, *Rumex obtusifolius*, *Pilea pumila*. Tout au moins ces espèces le sont plus que le *Laportea canadensis* et le *Circaea latifolia*, des nitrophiles authentiques, mais aussi des sciophiles. D'autre part le progrès des *Rumex Acetosella*, *R. obtusifolius*, *Dennstaedtia punctilobula* s'explique sans doute fort bien par les facteurs lumière et réaction du sol. De sorte que la meilleure qualité chimique de l'humus est en quelque sorte compensée par sa mauvaise condition physique que peuvent seules utiliser des espèces pionnières résistantes à des excès de lumière, d'humidité, de sécheresse ou d'acidité.

L'*Aceretum saccharophori pascuorum* se présentera donc sous une multitude de faciès différents: ce milieu souvent hétérogène pourra consister en une mosaïque de biotopes dont je décrirai ci-après quelques-uns.

*Faciès dénudé.* Dans une ombre encore très dense, quoique sans le bénéfice d'une strate arbustive proprement dite, il reste quelques *Sambucus pubens*. L'union herbacée a une couverture totale presque négligeable. Le *Dryopteris*

TABLEAU III

LES QUASICLIMAX, LE CLIMAX ET LES DISCLIMAX

	Association	Pente	Drainage	Température	Union de la synusie arborescente (mégaphanérophytes)
QUASICLIMAX NORDIQUES	8 <i>Aceretum saccharophori betulosum</i>	0-50°	Médiocre	Froide	<i>Acer saccharophorum</i> <i>Betula lutea</i> <i>Abies balsamea</i>
	9 <i>Aceretum saccharophori tsugosum</i>	0-50°	Irrégulier	Froide	<i>Acer saccharophorum</i> <i>Fagus grandifolia</i> <i>Tsuga canadensis</i> <i>Acer rubrum</i> <i>Betula lutea</i>
QUASICLIMAX MERIDIONAUX	10 <i>Aceretum saccharophori ulmosum</i>	0-25°	Médiocre	Froide	<i>Acer saccharophorum</i> <i>Fagus grandifolia</i> <i>Tilia americana</i> <i>Fraxinus americana</i> <i>Ulmus americana</i>
	11 <i>Aceretum saccharophori quercosum</i>	0-35°	Rapide	Chaude	<i>Acer saccharophorum</i> <i>Quercus borealis</i> <i>Fagus grandifolia</i> <i>Acer rubrum</i> <i>Tilia americana</i>
	12 <i>Aceretum saccharophori caryosum</i>	0-35°	Bon	Chaude	<i>Acer saccharophorum</i> <i>Fagus grandifolia</i> <i>Carya ovata</i> <i>Carya cordiformis</i> <i>Quercus alba</i> <i>Quercus macrocarpa</i> <i>Prunus serotina</i>
	13 <i>Aceretum saccharophori nigroides</i>	0-35°	Bon	Chaude	<i>Acer saccharophorum</i> <i>Acer nigrum</i> <i>Fagus grandifolia</i> <i>Carya ovata</i> <i>Tilia americana</i>

TABLEAU III

LES QUASICLIMAX, LE CLIMAX ET LES DISCLIMAX

Unions de la synusie frutescente (mésophanérophytes)	Unions de la synusie frutescente (microphanérophytes et chaméphytes)	Unions de la synusie herbacée haute	Unions de la synusie herbacée basse
<i>Corylus cornuta</i>	<i>Viburnum lantanoides</i> ↑	<i>Taxus canadensis</i>	<i>Lycopodium lucidulum</i> <i>Oxalis montana</i> ↑
↓	<i>Lonicera canadensis</i>		<i>Trientalis borealis</i> <i>Trillium undulatum</i> <i>Lycopodium obscurum</i>
<i>Acer spicatum</i>		<i>Ribes glandulosum</i>	<i>Dryopteris marginalis</i> <i>Hepatica acutiloba</i> ↓
			<i>Dryopteris spinulosa</i> <i>Maianthemum canadense</i> <i>Clintonia borealis</i>
<i>Corylus cornuta</i>	<i>Viburnum lantanoides</i>	<i>Osmunda cinnamomea</i> ↓	<i>Dryopteris spinulosa</i> <i>Maianthemum canadense</i> ↑
↓		<i>Taxus canadensis</i>	<i>Trientalis borealis</i> <i>Trillium undulatum</i> <i>Lycopodium obscurum</i> ↑
<i>Acer spicatum</i>			<i>Mitchella repens</i> <i>Cypripedium acaule</i> <i>Medeola virginiana</i>
<i>Acer pennsylvanicum</i> <i>Ostrya virginiana</i> <i>Corylus cornuta</i>	<i>Sambucus pubens</i> <i>Dirca palustris</i>	<i>Laportea canadensis</i> <i>Adiantum pedatum</i>	<i>Arisaema atrorubens</i> ↓ <i>Mitella diphylla</i> <i>Viola canadensis</i>
<i>Ostrya virginiana</i> <i>Carpinus caroliniana</i> ↑			<i>Uvularia sessilifolia</i> <i>Hepatica americana</i> ↓ <i>Trientalis borealis</i> <i>Lycopodium obscurum</i>
<i>Corylus cornuta</i>			
<i>Acer pennsylvanicum</i> <i>Cornus alternifolia</i>  <i>Hamamelis virginiana</i>	<i>Sambucus pubens</i>	<i>Osmorhiza Claytoni</i> <i>Desmodium grandiflorum</i> <i>Parthenocissus quinquefolia</i> <i>Smilax herbacea</i>	<i>Hydrophyllum virginianum</i> <i>Trillium grandiflorum</i> <i>Uvularia grandiflora</i> <i>Amphicarpaea bracteata</i>
<i>Acer pennsylvanicum</i> <i>Cornus alternifolia</i>	<i>Sambucus pubens</i>	<i>Osmorhiza Claytoni</i> <i>Desmodium grandiflorum</i>	<i>Mitella diphylla</i> <i>Viola canadensis</i> <i>Viola eriocarpa</i>

TABLEAU III—*fin*LES QUASICLIMAX, LE CLIMAX, ET LES DISCLIMAX—*fin*

	Association	Pente	Drainage	Température	Union de la synusie arborescente (mégaphanérophyles)
CLIMAX	14 <i>Aceretum saccharophori laurentianum</i>	0-35°	Bon	Fraîche	<i>Acer saccharophorum</i> <i>Fagus grandifolia</i> <i>Fraxinus americana</i>
	15 <i>Aceretum saccharophori dennstaediosum</i>	0-50°	Irrégulier	Fraîche, chaude	<i>Acer saccharophorum</i>
DISCLIMAX	16 <i>Aceretum saccharophori glyceriosum</i>	0-25°	Déficient	Froide, fraîche	<i>Acer saccharophorum</i> <i>Acer rubrum</i> <i>Betula lutea</i>
	17 <i>Aceretum saccharophori acerosum</i>	0-25°	Bon	Chaude	<i>Acer saccharophorum</i>
	18 <i>Aceretum saccharophori pascuorum</i>	0-35°	Déficient	Chaude	<i>Acer saccharophorum</i>

TABLEAU III—fin

LES QUASICLIMAX, LE CLIMAX, ET LES DISCLIMAX—fin

Unions de la synusie frutescente (mésophanérophytes)	Unions de la synusie frutescente (microphanérophytes et chaméphytes)	Unions de la synusie herbacée haute	Unions de la synusie herbacée basse
<i>Acer pennsylvanicum</i> <i>Cornus alternifolia</i>	<i>Sambucus pubens</i>	<i>Osmorhiza Claytoni</i> <i>Desmodium grandiflorum</i> <i>Eupatorium urticaefolium</i>	<i>Viola eriocarpa</i> <i>Mitella diphylla</i> <i>Carex arctata</i>
	<i>Prunus virginiana</i>	<i>Rubus</i> spp.	<i>Dennstaedtia punctilobula</i> ↑ <i>Danthonia spicata</i> <i>Antennaria neodioica</i> <i>Fragaria virginiana</i> <i>Hieracium aurantiacum</i> <i>Prunella vulgaris</i>
			<i>Dryopteris noveboracensis</i> <i>Dennstaedtia punctilobula</i> <i>Hieracium vulgatum</i> ↓ <i>Dryopteris spinulosa</i> ↓ <i>Maianthemum canadense</i> <i>Clintonia borealis</i> ↑ <i>Viola pallens</i> ↑ <i>Coptis groenlandica</i> ↑ <i>Calliergonella Schreberi</i> ↑ <i>Dicranum undulatum</i> ↑ <i>Glyceria striata</i> <i>Carex intumescens</i> <i>Onoclea sensibilis</i> <i>Impatiens biflora</i>
		<i>Acer saccharophorum</i> (jeune)	<i>Acer saccharophorum</i> (plantules)
	<i>Sambucus pubens</i>	<i>Equisetum hyemale</i>	<i>Geum canadense</i> <i>Hieracium vulgatum</i> ↑ <i>Glyceria striata</i> <i>Carex intumescens</i>
<i>Corylus cornuta</i> ↑		<i>Osmunda cinnamomea</i> <i>Carex crinita</i> ↑ <i>Osmunda regalis</i> ↑ <i>Veratrum viride</i>	
<i>Alnus incana</i>	<i>Cornus stolonifera</i>		<i>Dryopteris noveboracensis</i> ↑ <i>Dennstaedtia punctilobula</i> ↑ <i>Hieracium vulgatum</i> ↑ <i>Galeopsis Tetrahit</i> ↑ <i>Phleum pratense</i> <i>Taraxacum officinale</i> <i>Antennaria neodioica</i>
	<i>Prunus virginiana</i>	<i>Verbascum Thapsus</i>	

TABLEAU IV

EFFET DE L'AUGMENTATION OU DE LA DIMINUTION DE LA LUMIÈRE, DE LA CHALEUR, DE L'HUMIDITÉ ET DU pH SUR DIVERSES ESPÈCES

		Augmentation	Stabilité	Diminution
LUMIÈRE ACCRUE	Espèces du climax	<i>Carex pedunculata</i> <i>Aralia racemosa</i> <i>Erythronium americanum</i> <i>Trillium grandiflorum</i>	<i>Carex plantaginea</i> <i>Actaea</i> spp. <i>Claytonia caroliniana</i> <i>Carex arctata</i>	<i>Viola canadensis</i> <i>Osmorhiza Claytoni</i> <i>Asarum canadense</i> <i>Hydrophyllum virginianum</i> <i>Solidago latifolia</i> <i>Solidago caesia</i> <i>Dileprium erectum</i> <i>Sanguinaria canadensis</i> <i>Adiantum pedatum</i> <i>Dicentra</i> spp. <i>Ranunculus abortivus</i> <i>Amphicarpaea bracteata</i>
	Espèces des quasi-climax et des sous-climax	<i>Viola papilionacea</i> <i>Carex albursina</i> <i>Lycopodium complanatum</i> <i>Aralia nudicaulis</i> <i>Maianthemum canadense</i> <i>Aster macrophyllus</i> <i>Gaultheria procumbens</i> <i>Vitis vulpina</i> <i>Veratrum viride</i> <i>Poa nemoralis</i>	<i>Onoclea sensibilis</i>	<i>Circaea alpina</i> <i>Linnaea borealis</i> <i>Chiogenes hispidula</i>
	Espèces pionnières	<i>Lycopodium clavatum</i> <i>Dryopteris cristata</i> <i>Agrimonia gryposepala</i> <i>Danthonia spicata</i> <i>Thalictrum polygamum</i> <i>Geum rivale</i> <i>Fragaria virginiana</i> <i>Aster cordifolius</i> <i>Anaphalis margaritacea</i> <i>Achillea Millefolium</i> <i>Aster nemoralis</i> <i>Poa pratensis</i> <i>Polygonum Persicaria</i> <i>Geranium Robertianum</i> <i>Trifolium repens</i> <i>Nepeta Cataria</i> <i>Ranunculus acris</i> <i>Silene Cucubalus</i> <i>Stellaria media</i> <i>Stellaria longifolia</i> <i>Taraxacum officinale</i> <i>Leonurus Cardiaca</i> <i>Erigeron philadelphicus</i> <i>Hieracium aurantiacum</i> <i>Hieracium vulgatum</i> <i>Galeopsis Tetrahit</i> <i>Apocynum androsaemifolium</i> <i>Rubus idaeus</i> <i>Prunella vulgaris</i> <i>Polygonum Convolvulus</i> <i>Solidago canadensis</i> <i>Solidago rugosa</i> <i>Antennaria neodioica</i> <i>Verbascum Thapsus</i> <i>Dennstaedtia punctilobula</i> <i>Epilobium angustifolium</i> <i>Pteridium latiusculum</i>		<i>Lycopus uniflorus</i> <i>Equisetum sylvaticum</i>
CHALEUR ACCRUE	Espèces du climax	<i>Carex pedunculata</i>	<i>Carex arctata</i>	<i>Galium triflorum</i> <i>Tiarella cordifolia</i> <i>Dryopteris spinulosa</i> <i>Lycopodium lucidulum</i>
	Espèces des quasi-climax et des sous-climax	<i>Parthenocissus quinquefolia</i> <i>Galium lanceolatum</i> <i>Urtica sessilifolia</i>	-	<i>Clintonia borealis</i> <i>Oxalis montana</i> <i>Pyrola secunda</i> <i>Cornus canadensis</i> <i>Ribes glandulosum</i> <i>Lycopodium annotinum</i> <i>Solidago macrophylla</i> <i>Maianthemum canadense</i> <i>Viola pallens</i>
	Espèces pionnières	<i>Aquilegia canadensis</i>		

TABLEAU IV—fin

EFFET DE L'AUGMENTATION OU DE LA DIMINUTION DE LA LUMIÈRE, DE LA CHALEUR, DE L'HUMIDITÉ, ET DU pH SUR DIVERSES ESPÈCES—fin

		Augmentation	Stabilité	Diminution
HUMIDITÉ ACCRUE	Espèces du climax	<i>Rubus pubescens</i> <i>Arisaema atrorubens</i> <i>Adiantum pedatum</i>	<i>Hydrophyllum virginianum</i>	
	Espèces des quasi-climax et des sous-climax	<i>Chiogenes hispidula</i> <i>Linnaea borealis</i> <i>Viola sororia</i> <i>Panax trifolium</i> <i>Viola septentrionalis</i> <i>Coptis groenlandica</i> <i>Laportea canadensis</i> <i>Maianthemum canadense</i> <i>Athyrium angustum</i> <i>Dryopteris marginalis</i> <i>Trillium undulatum</i> <i>Geum canadense</i> <i>Viola renifolia</i> <i>Circaea alpina</i> <i>Osmunda cinnamomea</i> <i>Impatiens biflora</i> <i>Viola cucullata</i> <i>Onoclea sensibilis</i> <i>Symplocarpus foetidus</i> <i>Veratrum viride</i> <i>Carex intumescens</i> <i>Viola blanda</i>	<i>Mitchella repens</i>	<i>Aralia nudicaulis</i> <i>Monotropa uniflora</i> <i>Corallorrhiza maculata</i>
HUMIDITÉ DIMINUÉE	Espèces du climax	<i>Thalictrum dioicum</i> <i>Uvularia grandiflora</i>	<i>Carex arctata</i> <i>Smilacina racemosa</i>	<i>Viola eriocarpa</i> <i>Asarum canadense</i> <i>Mitella diphylla</i> <i>Trillium erectum</i> <i>Polygonatum pubescens</i> <i>Adiantum pedatum</i> <i>Allium tricoccum</i>
	Espèces des quasi-climax et des sous-climax	<i>Carex albursina</i> <i>Dryopteris noveboracensis</i> <i>Gaultheria procumbens</i> <i>Trientalis borealis</i> <i>Dalibarda repens</i> <i>Medeola virginiana</i> <i>Uvularia sessilifolia</i>		
	Espèces pionnières	<i>Fragaria virginiana</i> <i>Aster cordifolius</i> <i>Anaphalis margaritacea</i> <i>Verbascum Thapsus</i> <i>Aquilegia canadensis</i> <i>Dennstaedtia punctilobula</i> <i>Arctium Lappa</i> <i>Silene Cucubalus</i>		
	Espèces du climax		<i>Polygonatum pubescens</i> <i>Trillium erectum</i> <i>Erythronium americanum</i>	<i>Allium tricoccum</i> <i>Mitella diphylla</i> <i>Arisaema atrorubens</i> <i>Epipactis latifolia</i> <i>Eupatorium urticaefolium</i> <i>Dicentra Cucullaria</i>
pH ABAISSÉ	Espèces des quasi-climax et des sous-climax	<i>Mitchella repens</i> <i>Lycopodium obscurum</i> <i>Pyrola elliptica</i> <i>Cornus canadensis</i> <i>Chimaphila umbellata</i> <i>Oxalis montana</i> <i>Clintonia borealis</i> <i>Aster acuminatus</i> <i>Dalibarda repens</i> <i>Maianthemum canadense</i> <i>Medeola virginiana</i> <i>Trillium undulatum</i> <i>Siretiopus roseus</i>		<i>Laportea canadensis</i> <i>Circaea latifolia</i>
	Espèces pionnières	<i>Lycopodium complanatum</i> <i>Oryzopsis pungens</i> <i>Danthonia spicata</i> <i>Fragaria virginiana</i> <i>Anaphalis margaritacea</i> <i>Pteridium latiusculum</i> <i>Rubus idaeus</i> <i>Silene Cucubalus</i>		<i>Trifolium repens</i> <i>Geranium Robertianum</i> <i>Saxifraga virginianensis</i>



*spinulosa* et le *Carex arctata* (espèces du climax) ont aussi persisté, et le *Ranunculus abortivus* maintient sa position à la fourche des racines des plus gros arbres. Une certaine congestion dans le drainage favorise l'*Oxalis montana* et le *Polytrichum commune*, de même que quelques autres mousses; de même le *Geum canadense* et l'*Equisetum hyemale* se trouvent bien du tassement du sol. On rencontre aussi quelques rares individus des plus tolérantes parmi les mauvaises herbes, telles les *Galeopsis Tetrahit*, *Hieracium vulgatum* et *Taraxacum officinale*. Ces dernières sont parfois affectées dans leurs caractères morphologiques, au point d'être difficiles à reconnaître. La régénération des espèces arborescentes est nulle.

*Faciès humide.* Avec une diminution de l'ombre accompagnée d'une augmentation de l'humidité du sol (tendance exprimée par l'*Aceretum saccharophori glyceriosum*, no 16) une autre série d'espèces est favorisée. Les quelques arbustes qui pourront survivre seront des *Corylus cornuta* ou des *Thuja occidentalis*. Les plantes herbacées seront réparties dans divers biotopes plus ou moins zonés selon le degré et la durée de la saturation d'eau du sol: a) biotope des hygrophiles typiques (*Onoclea sensibilis*, *Glyceria striata*, *Carex intumescens*); b) biotope des nitrophiles (*Pilea pumila*, *Circaea latifolia*, *Laportea canadensis*); c) biotope des mésophiles particulièrement résistantes (*Panax trifolium*, *Agrostis stolonifera*, *Athyrium angustum*, *Equisetum sylvaticum*). Une pareille composition botanique suppose un relief inégal et la formation de flaques plus ou moins isolées et ne séchant que très lentement au début de l'été. Les trois séries de plantes mentionnées plus haut présenteront donc une zonation assez nette depuis le fond des dépressions jusque sur les buttes au pied des arbres.

*Faciès palustre.* Sur un sol encore plus humide (et souvent plus éclairé), il se constituera une union arbustive clairsemée à *Alnus incana* et *Cornus stolonifera*. La synusie herbacée abritera une suite de biotopes: le fond des dépressions contiendra le *Chrysosplenium americanum* et le *Caltha palustris*; auquel feront suite le *Veratrum viride*, le *Symplocarpus foetidus*, l'*Osmunda regalis*, le *Carex crinita*; puis le *Geum rivale*, l'*Osmunda cinnamomea*, l'*Impatiens biflora*, et enfin des espèces plus mésiques et quelques mousses. L'été, au cours d'un assèchement relatif, il peut se constituer dans ces fonds une mégaphorbiée à deux unions, l'une à *Veratrum viride*, *Eupatorium perfoliatum*, *Aster umbellatus*, et l'autre, inférieure, à *Mentha canadensis*, *Lycopus uniflorus*, *Lysimachia terrestris*.

*Faciès sec.* Dans une ombre réduite, mais sur un sol à drainage excessif ou irrégulier, le *Prunus virginiana* pourra survivre; le *Rubus allegheniensis*, de même. Tandis que des espèces résistantes à la fois à la sécheresse et à une ombre partielle s'empareront de la plus grande partie du terrain. Le biotope à *Dennstaedtia punctilobula* et *Dryopteris noveboracensis* surtout pourra être très étendu; tandis que des individus isolés ou de petites colonies d'autres espèces seront éparpillés ici et là: *Uvularia sessilifolia* (voir l'*Aceretum saccharophori quercosum*, no 11), *Rumex Acetosella*, *Taraxacum officinale*, *Achillea Millefolium*, *Hieracium vulgatum*, *Veronica officinalis*, *Cinna latifolia*,

*Oryzopsis pungens*, *Galeopsis Tetrahit*, *Erigeron philadelphicus*, *Geranium Robertianum*. Il faut peut-être signaler que ces espèces sont plus ou moins acidophiles. Leur résistance à l'aridité et leurs exigences notoirement faibles les rendent particulièrement aptes à occuper un tel biotope. De même les espèces caractéristiques de l'*Aceretum saccharophori tsugosum* (no 9) se rencontreront fréquemment ici.

*Faciès champêtre.* Un dernier type physionomique, qui n'appartient pratiquement plus au milieu sylvestre tant il est caractéristique d'une dégradation avancée, est contrôlé dans sa composition par une extrême héliophilie. La reproduction des érables, hêtres et autres mésophytes était impossible dans les cas précédents; ici ce sont plutôt les bouleaux, trembles et aubépines (*Crataegus* spp.) qui commenceront à se régénérer. Toutes les plantes herbacées, à peu près sans exception, seront des 'mauvaises herbes' ou tout au moins des espèces introduites (Dansereau, 1943 (14), Tableau IX). Ce faciès est une prairie ou une pelouse à l'ombre claire de vieux érables. Ces herbes seront, entre autres, les *Phleum pratense*, *Taraxacum officinale*, *Trifolium repens*, *Poa compressa*, *Poa pratensis*, *Hieracium aurantiacum*, *Antennaria neodioica*, *Verbascum Thapsus*, *Potentilla argentea*.

### Les proclimax

Cette notion proposée par Clements et plusieurs fois redéfinie par lui (voir Clements, 1936 (11)) remplace tout le motif ('pattern') des successions de la forêt décidue laurentienne dans son cadre géographique et demande qu'on tienne compte de la zonation, de l'orientation, et de l'évolution de la clisère tout entière. Dans deux communications précédentes (Dansereau, 1944 (15, 16)), j'ai esquissé les grandes lignes de ces relations à la fois floristiques et bioclimatiques, et il m'a semblé pouvoir affirmer, en conclusion préliminaire et d'accord avec plusieurs autres biogéographes, que le climat actuel de la Vallée du Saint-Laurent allait se refroidissant. Je me réserve de traiter ce sujet plus en détail (voir plus haut, pages 257 et 263).

Mais je crois devoir signaler ici l'équivalence foncière qui existe entre les préclimax et postclimax et les quasiclimes décrits plus haut. Ainsi, dans la région de Québec, on ne rencontre pratiquement plus le climax pur (no 14), mais ordinairement (et ce sur la meilleure topographie) l'*Aceretum saccharophori betulosum* (no 8). Devons-nous considérer cette association comme préclimax du *Piceetum marianae* environnant ou comme postclimax de l'*Aceretum saccharophori laurentianum* qui domine vers le sud-ouest? Avant de résoudre pareille question, et bien d'autres que la présente étude soulève, il faudra faire intervenir beaucoup de données quantitatives, passant les quelque 500 relevés faits à date dans des érablières laurentiennes au crible d'un test des indicateurs floristiques et écologiques afin de mieux connaître les gradients de la variation géographique et le jeu des facteurs compensateurs.

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## Appendice

Je crois bon d'ajouter ici la définition de quelques termes que Carpenter dans sa compilation (*An Ecological Glossary*. Kegan Paul, Trubner, Trench, Londres, 1938) a insuffisamment traités ou n'a pas mentionnés.

*Climax*: association d'une composition botanique et d'une structure définies, d'un équilibre autodéterminé et d'un pouvoir d'utilisation maximum qui occupe la topographie la plus favorable sur toute l'étendue géographique et au cours de la durée entière d'un cycle climatique.

*Quasiclimax*: association qui contient au moins l'une des dominantes ou co-dominantes du climax, mais ne peut évoluer, au cours du cycle climatique, jusqu'à la maturité finale, à cause d'une inhibition locale topographique ou microclimatique.

*Sous-climax*: association de structure semblable à celle du climax, mais dont la composition botanique permet une évolution ultérieure.

*Disclimax*: sous-climax ou association pionnière dont la permanence est due à la répétition périodique d'une même intervention.

*Pionnière*: association du début de la sère dont les formes biologiques dominantes sont autres que celles du climax et des sous-climax.

*Proclimax*: association relique d'un climat antérieur plus sec et plus chaud (*préclimax*) ou plus froid et plus humide (*postclimax*).

*Faciès*: variante d'une association où une ou des espèces habituellement subdominantes ou commensales deviennent dominantes dans l'une ou l'autre des synusies.

*Union*: communauté de composition botanique définie occupant une synusie. Une association se compose d'une ou de plusieurs unions.

*Biotope*: conditions immédiates de vie pour une espèce en particulier: écorce d'un arbre pour un lichen, feuilles d'une plante herbacée pour un champignon, petite dépression humide pour une mousse.—N.B. Il ne peut être question de biotope pour un érable, par exemple, si ce n'est au stade de graine ou de plantule.

## Summary

A sketch is presented of the broad lines of succession in the deciduous forest area of the St. Lawrence Valley. The climax is a deciduous forest, the *Aceretum saccharophori laurentianum* in which *Acer saccharophorum* is dominant. Such a terminal point of the evolution of regional vegetation is attained through five principal channels.

In the course of succession, a gradual improvement of the site takes place through pioneer and subclimax associations. The latter are described summarily in their floristic dominants and their phytosociological rôle.

Moreover, the diverse successions often include a subterminal stage very close to the climax, to which it conforms in most essential characteristics, but in which further evolution is checked by a topographic or micro-climatic cause and where elements persist that are typical of the sere from which it is derived. These quasiclimaxes and the climax itself are described in some detail as well as the unions that characterize their structure.

The prisere having thus been defined, a few elements of the subsere are in turn analysed: the maple groves where human intervention has taken place or is still active.

These disclimaxes are essentially of two types according to whether degradation is due to lumbering or to pasture. Conditions unknown in the prisere are realized here which permit the establishment of new units, according to the capacity of adaptation of species. These associations, faciès, unions, or biotopes naturally owe their duration to the more or less regular repetition of man's interference.

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## AWN-BARBING IN BARLEY<sup>1</sup>

BY H. A. FRIESEN<sup>2</sup>

### Abstract

The inheritance of awn-barbing was studied in crosses of barley varieties having intermediate smooth and very smooth awns and lacking the gene *R* for rough awns. Two genes were found to govern awn smoothness in these varieties, one being *S* hypostatic to *R* as previously reported and the other, termed *S*<sub>1</sub>, hypostatic to *S*. The genotype *rrSSS*<sub>1</sub>*S*<sub>1</sub> has intermediate smooth awns, *rrssS*<sub>1</sub>*S*<sub>1</sub> has smooth awns, and *rrsss*<sub>1</sub>*S*<sub>1</sub> has very smooth awns.

### Introduction

While the inheritance of awn-barbing in barley has been studied by a number of workers, their results, in almost all cases, indicated that a more critical study would present a clearer picture of the factors involved. Since large scale breeding programs to produce smooth awned barley varieties are being actively carried on, it was felt that a more intensive study of the inheritance of awn-barbing would yield information valuable to the breeder.

### Literature Review

The literature on awn-barbing in barley is fairly extensive and shows the existence of two genes governing that character. Hayes *et al.* (2) explained the inheritance of awn-barbing in a cross of Lion × Manchuria on the basis on one main dominant gene for rough awns, plus modifiers. Griffie (1) using the cross Lion × Svanhals reported two dominant genes, *R* for rough awns and *S* for intermediate smooth awns with *R* being epistatic to *S* and the *F*<sub>2</sub> phenotypic ratio of 12 : 3 : 1 of rough, intermediate, and smooth being confirmed by *F*<sub>3</sub> progeny tests. A similar explanation was given by Robertson *et al.* (4) for the results obtained from the cross Coast × Lion and by Johnston and Aamodt (3) for their results with Velvet × Trebi and Glabron × Trebi. Sigfusson (5) explained his results from Bearer × Lion as being due to two complementary factors.

### Materials and Methods

Six varieties of barley were sown in the cereal nursery at Saskatoon for observation. It was found that at least four types of awn-barbing were present. These were rough, intermediate, smooth, and very smooth. The varieties bred true for the type that they possessed. O.A.C. 21, a Manchurian selection, is a representative rough awned variety. Velvet, Regal, and Newal are typically smooth. This type has rudimentary barblets covering the

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upper quarter of the awn. The intermediate type has barbs, reduced in size to a point intermediate between rough and smooth awns, over the upper 50 to 75% of the awn. The variety O.P.R. 1, a selection from the triple cross (O.A.C. 21  $\times$  Peatland)  $\times$  Regal, breeds true for intermediate awn-barbing. The fourth type of awn-barbing, termed 'very smooth' is found in the variety Velvon from the cross (Lion  $\times$  Coast)  $\times$  Trebi. In this type the awn is usually free of barbs throughout its entire length; however, a few very rudimentary barblets may be found near the tip. Some variation in the degree of smoothness was found to exist within the three types showing some smoothness of awn. This variation was found between different plants, between different tillers, and between different awns on the same head.

A number of crosses were made between varieties possessing intermediate smooth and smooth awns and between those having intermediate smooth and very smooth awns. In addition three intravarietal crosses were made, using the varieties Velvet, Newal, and Regal, to determine whether the slight variations in awn-smoothness within a variety could be due to genetic differences. The crosses within each variety were made between plants exhibiting the widest variations in the degree of awn-smoothness.

The classification of awn-barbing was done by two methods. The first method used was the simple method of 'feel,' the awns being classified by their feel when drawn between the thumb and forefinger. For more accurate work, binoculars were used, all the awns of the head being examined and the phenotypic class determined by comparison with the parental varieties. This procedure was very slow. A combination of the two methods was used for more rapid work, the awns being tested for awn-barbing class by feel, and only those plants that were difficult to classify were checked by the use of the binoculars. The low power of a microscope was also used in classifying plants that seemed to lie between the smooth and the very smooth classes.

The need of growing the  $F_1$ ,  $F_2$ , and  $F_3$  in only one year's time required the  $F_2$  and  $F_3$  to be grown in the greenhouse. Owing to the limitation of space only a relatively small proportion of the  $F_2$  plants were given progeny tests in  $F_3$  but the number tested proved to be sufficient.

### Experimental Results

The results from the classification of the  $F_1$  and  $F_2$  plants for awn-barbing are given in Table I.

#### *O.P.R. 1 $\times$ Velvet*

The  $F_2$  segregation observed in this cross was 94 intermediate and 38 smooth awned plants. A goodness of fit test applied to the results on the basis of a 3 : 1 ratio of intermediate to smooth awned gave a  $\chi^2$  of 1.01 and a  $P$  value of .3 thus showing a single factor-pair to be responsible for the awn-barbing differences observed. Thirty-four  $F_3$  families were grown from the  $F_2$  plants. The  $F_2$  results corrected on the basis of the  $F_3$  progeny tests showed eight progeny breeding true for intermediate, 15 segregating, and 11 breeding true



TABLE I

CLASSIFICATION OF THE  $F_1$  AND  $F_2$  OF A NUMBER OF BARLEY  
CROSSES FOR BARBING OF THE AWNS

Cross	$F_1$	$F_2$ plants		
		Intermediate	Smooth	Very smooth
O.P.R. 1 $\times$ Velvet	Intermediate	94	38	—
O.P.R. 1 $\times$ Velvet	Intermediate	235	63	24
Velvet $\times$ Velvet	Smooth	2	111	3
Newal $\times$ Newal	Smooth	4	167	4
Regal $\times$ Regal	Smooth	8	190	2

for smooth. A goodness of fit test applied to these results on the basis of a 1 : 2 : 1 ratio gave a  $\chi^2$  of 1.12 and a  $P$  value of .5 clearly indicating the action of a single pair of factors. In Table II the 15 segregating  $F_3$  families were each tested for their goodness of fit to a ratio of 3 intermediate : 1 smooth. The  $P$  value of less than .05 for the recessive class of Family 6 is low but as no other family shows a  $P$  value below .05 there is little reason to feel that the poor distribution in this family is genetical.

TABLE II

TEST OF AGREEMENT BETWEEN OBSERVED AND EXPECTED CLASS FREQUENCIES APPLIED TO  
15  $F_3$  FAMILIES OF THE CROSS O.P.R. 1  $\times$  VELVET, ON THE HYPOTHESIS OF  
A 3 : 1 MENDELIAN SEGREGATION FOR INTERMEDIATE  
AND SMOOTH AWNS, RESPECTIVELY

No. of family	Observed	Expected	Chi square $\chi^2$	$P$ value
1	28 : 8	27 : 9	0.148	.7
2	15 : 6	15.75 : 5.25	0.143	.7
3	12 : 4	12 : 4	0.000	.99
4	21 : 5	19.5 : 6.5	0.461	.5
5	24 : 12	27 : 9	1.333	.3
6	30 : 18	36 : 12	4.000	.05
7	15 : 6	15.75 : 5.25	0.143	.7
8	24 : 6	22.5 : 7.5	0.400	.5
9	24 : 8	24 : 8	0.000	.99
10	28 : 12	30 : 10	0.533	.5
11	29 : 13	31.5 : 10.5	0.793	.5
12	21 : 5	19.5 : 6.5	0.461	.5
13	29 : 15	33 : 11	1.940	.2
14	21 : 12	25.5 : 8.5	1.921	.2
15	22 : 4	19.5 : 6.5	1.283	.3
$\chi^2 = 13.560$		$n = 15$	$P = .5$	

### *O.P.R. 1 $\times$ Velvet*

The data from classifying the  $F_1$  and  $F_2$  of this cross are given in Table I. Seventy-three  $F_2$  plants were given  $F_3$  progeny check ups. The  $\chi^2$  test for the  $F_2$  fit to a 12 : 3 : 1 ratio of intermediate, smooth, and very smooth gave a

$\chi^2$  of 1.035 and a  $P$  value of .5 clearly showing that the difference observed in awn-barbing between O.P.R. 1 and Velvon is determined by two factor-pairs. Table III gives the results of the studies on the  $F_2$  families of this cross. In Table IV the  $F_2$  breeding classes are tested for their goodness of fit to the ratio expected on the basis of two factor-pairs determining the types of awn-barbing observed.

TABLE III

SUMMARY OF AWN-BARBING BEHAVIOUR OF 73  $F_2$  FAMILIES FROM THE CROSS  
O.P.R. 1  $\times$  VELVON

$F_2$ plants, genotype	Number	Theoretical segregation of $F_2$ families			Observed segregation in all the $F_2$ families		
		Inter- mediate	Smooth	Very smooth	Inter- mediate	Smooth	Very smooth
$rrSSS_1S_1$ , $rrSSS_1s_1$ , and $rrSSs_1s_1$	21	All	—	—	196	4	—
$rrSsS_1S_1$	11	3	1	—	79	33	—
$rrSsS_1s_1$	16	12	3	1	113	24	6
$rrSSs_1s_1$	9	3	—	1	62	7	16
$rrssS_1S_1$	8	—	All	—	6	69	1
$rrssS_1s_1$	5	—	3	1	4	39	9
$rrssss_1s_1$	3	—	—	All	—	3	26

TABLE IV

FREQUENCIES OF  $F_2$  BREEDING CLASSES OF THE CROSS O.P.R. 1  $\times$  VELVON, COMPARED WITH THE  
THEORETICAL 4 : 2 : 4 : 2 : 1 : 2 : 1 RATIO ON THE BASIS OF A TWO-FACTOR  
DIFFERENCE BETWEEN INTERMEDIATE AND VERY SMOOTH AWNS

$F_2$ plants, genotype	Number of $F_2$ plants		$O - C$	$(O - C)^2$	$\frac{(O - C)^2}{C}$
	Observed (O)	Expected (C)			
4 $\left\{ \begin{array}{l} rrSSS_1S_1 \\ rrSSS_1s_1 \\ rrSSs_1s_1 \end{array} \right.$	21	18.252	2.748	7.5515	0.414
$2rrSsS_1S_1$	11	9.126	1.874	3.5119	0.385
$4rrSsS_1s_1$	16	18.252	-2.252	5.0715	0.278
$2rrSSs_1s_1$	9	9.126	-0.126	0.1588	0.017
$1rrssS_1S_1$	8	4.563	3.437	11.8130	2.589
$2rrssS_1s_1$	5	9.126	-4.126	17.0239	1.865
$1rrssss_1s_1$	3	4.563	-1.563	2.4430	0.535
$\chi^2 = 6.083$		$P = .4$			

The results given in Table III show that the segregation of plants within the  $F_2$  families was fairly close to expectation, especially in the intermediate class. In the smooth and very smooth classes there was a slight tendency to classify some of the smooth awned plants as intermediate and very smooth, while some very smooth plants were classified as smooth.

The  $P$  value of .4 given in Table IV clearly shows that the difference in awn-barbing between O.P.R. 1 and Velvon is determined by two pairs of factors.

*Velvet*  $\times$  *Velvet*

*Newal*  $\times$  *Newal*

*Regal*  $\times$  *Regal*

The results from the classification of the  $F_1$  and  $F_2$  of these three intra-variety crosses are given in Table I. From the  $F_2$  results it is apparent, without the use of statistical analyses, that the variations in smoothness of awn in these varieties were not caused by a difference in genotype. The slight tendency to call some of the plants intermediate indicates as in the previous crosses of O.P.R. 1  $\times$  Velvet and O.P.R. 1  $\times$  Velvon the possibility of the presence of a gene modifying the degree of awn smoothness.

### Discussion

Investigations by Johnston and Aamodt (3) and others have shown that the difference between the rough and intermediate awn-barbing types is determined by the single factor pair  $Rr$ ,  $R$  being a dominant gene for roughness of awn. They also found that a second factor pair  $Ss$  explained the difference between intermediate and smooth awned types. The factor  $S$  hypostatic to  $R$  produced the intermediate condition; while  $s$ , allelomorphic to  $S$ , when homozygous produced the smooth awned condition.

The results of the present study indicate that the difference between the intermediate and very smooth awned types is determined by two factor pairs. The factor  $S$  again produces intermediate awn-barbing and is partially dominant to its allelomorph  $s$ . The other factor termed  $S_1$  is hypostatic to  $S$ , while its allelomorph,  $s_1$ , when homozygous produces the very smooth awned condition. Thus a total of three factor-pairs are responsible for the awn-barbing types that would be observed in a cross of rough by very smooth awned varieties. The ratio expected in the  $F_2$  from such a cross would be 48 : 12 : 3 : 1 of rough, intermediate, smooth, and very smooth awned types, respectively. Thus the  $F_2$  segregation expected in a cross of intermediate with very smooth would be 12 intermediate : 3 smooth : 1 very smooth awned, if three pairs of factors are involved. Typically, the following genotypes would indicate the corresponding phenotypes;  $RRSSS_1S_1$ , rough awns;  $rrSSS_1S_1$ , intermediate awns;  $rrssS_1S_1$ , smooth awns, and  $rrsss_1s_1$ , very smooth awns. Consequently the genotypes and breeding behaviours that would be expected in the  $F_3$  plants of a cross of O.P.R. 1  $\times$  Velvon would be:

	$F_2$ genotype	$F_3$ behaviour
	(1rrSSS <sub>1</sub> S <sub>1</sub> )	
	(2rrSSS <sub>1</sub> s <sub>1</sub> )	Four breeding true for intermediate
	(1rrSSs <sub>1</sub> S <sub>1</sub> )	
Intermediate	(4rrSsS <sub>1</sub> S <sub>1</sub> )	Four segregating in the ratio of 12 : 3 : 1 for intermediate: smooth: very smooth
	(2rrSsS <sub>1</sub> s <sub>1</sub> )	Two segregating in the ratio of 3 intermediate: 1 smooth
	(2rrSsss <sub>1</sub> s <sub>1</sub> )	Two segregating in the ratio of 3 intermediate: 1 very smooth

$F^2$ genotype	$F^2$ behaviour
Smooth $\left\{ \begin{array}{l} (1rrssS_1S_1) \\ (2rrssS_1S_1) \end{array} \right.$	One breeding true for smooth Two segregating in the ratio of 3 smooth: 1 very smooth
Very smooth $1rrssS_1s_1$	One breeding true for very smooth

The results on the two crosses O.P.R. 1  $\times$  Velvet and O.P.R. 1  $\times$  Velvon clearly show this hypothesis to be correct.

The near-smooth type of awn-barbing described by Johnston and Aamodt (3) as being somewhat less scabrous than the intermediate type could not be established with any degree of certainty in this study. Hence only the intermediate, smooth, and very smooth awned phenotypes were used. However, the slight variation in the intermediate type of awn-barbing indicates that the factor  $S$  is not completely dominant to  $s$ .

The slight variations observed in the smooth and very smooth awned types indicate the possible presence of one or more modifying factors. Since the effect of such a factor or factors is very slight and awn-barbing is also somewhat affected by environmental conditions, a genetic study of any such factor would be exceedingly difficult.

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## EFFECT OF HUMIDITY ON THE LONGEVITY OF *POPULUS* AND *ULMUS* SEEDS IN STORAGE<sup>1</sup>

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### Abstract

Storage of *Populus tremuloides* and *P. grandidentata* seeds at room temperature in a controlled-humidity series showed the optimum to be 20% relative humidity for both species. At this humidity level, seed viability was retained by *P. grandidentata* for 555 days and by *P. tremuloides* for 455 days, as compared to approximately 28 days for both checks (open air).

Similar storage of seeds of four *Ulmus* species showed tolerance of a wide humidity range by all species, the optimum storage condition being within a range of approximately 20 to 50% relative humidity.

Germinability in all four *Ulmus* species was greatly increased when seeds were tested in a lighted germinator.

### Introduction

Seeds of *Populus* stored under ordinary room conditions were found to lose viability within a few weeks. A survey of available literature revealed only the work of Faust (1), who described a method for storing *Populus* seeds that consisted, essentially, of predrying in open air for three to eight days followed by storage in closed bottles at 5° C. However, no humidity control was used in either the drying or bottle-storage phases of the method.

The work in *Populus* reported herein was begun in May, 1938, to obtain further information on seed longevity by studying the effects of storage in a humidity-control series. The work of Moss (4), which anticipated some aspects of the present study, appeared at about the same time.

Similar work in *Ulmus* was undertaken in 1940 and again in 1945 to obtain preliminary information on seed storage and germinative requirements.

### Materials and Methods

The 5-in. desiccators used as controlled-humidity chambers were stored, together with open-air check materials, in diffuse light in the laboratory at approximately 20° C. All germination tests were based on samples of 100 seeds and made on moist blotters at 25° C. The type of germinator varied with different experiments.

#### *Populus* Experiment

In May, 1938, seed-bearing catkins were collected from *P. tremuloides* Michx. and *P. grandidentata* Michx. as they began to fluff, and placed in the sunlight to complete seed dehiscence in trays protected by wire mesh screens.

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After one day in these trays the seeds were separated from the fluff and placed immediately in a series of desiccators maintained at constant relative humidities of 7, 20, 35, 52, 65, and 79%. The lowest of these humidities was obtained with calcium chloride\* and the higher humidities, following the method of Spencer (5, pp. 67, 68), with saturated solutions of potassium acetate, chromium oxide, sodium chromate, magnesium acetate, and ammonium chloride, respectively.

Germination tests were made at arbitrary intervals in a glass-topped, water-cooled germinator. Counts were made after three and six days. Weak seedlings, in which hypocotyl development was very slow or failed to raise the cotyledons from the blotter, were counted separately.

### *Ulmus Experiments*

For the 1940-41 experiment, seeds were collected from *U. americana* L., *U. laevis* Poll., and *U. pumila* L. as they began to fall from the trees, dried in the open air for one day, and placed in controlled-humidity storage as described for *Populus* seeds, except that the 65 and 79% relative humidity conditions were omitted.

For the 1945-46 experiment, seeds from *U. americana* L. and  $\times$  *U. hollandica* Mill. were prepared in the same way, but were stored under somewhat different humidity conditions (compare Tables III and IV).

Sulphuric acid concentrations, used to provide the different degrees of humidity, were based on the data of Wilson (6) and Moss (4), as follows: 64.5, 57.5, 50.7, 43.1, and 35.8% concentrated sulphuric acid (1.84 sp. gr.) were used to obtain 10, 20, 35, 50, and 65% relative humidities, respectively.

All routine germination tests in *Ulmus* were made at monthly intervals in a lighted germinator, previously described (2). The source of light was two 20 w. fluorescent tubes.

The germination tests in dark and light were made in 1940 on freshly collected seeds, and in 1945 on seeds stored for five months in the open air (check material).

## Experimental Results and Discussion

### *Populus Experiment*

Comparison of Tables I and II indicates that seeds of *P. tremuloides* tolerate a lower storage humidity than those of *P. grandidentata* (compare data for 7% humidity), and that seeds of *P. grandidentata* are more tolerant of the higher humidities (compare data for 52% and higher humidities).

Weak seedlings occur in about the same proportions among the germinating seedlings of both species. Moss (4) reported weak seedlings from a number of *Populus* and *Salix* species.

Results from both species agree in showing 20% relative humidity to be superior. At this humidity viability was retained by *P. grandidentata* seeds

\* The relative humidity produced by calcium chloride was determined by an Airguide humidity meter.

for 555 days and by *P. tremuloides* seeds for 455 days, as compared to 28 days for the checks.

TABLE I

PERCENTAGE SEED GERMINATION IN *Populus tremuloides* AFTER STORAGE  
AT DIFFERENT RELATIVE HUMIDITIES

Storage time, days	Relative humidity, %						
	7	20	35	52	65	79	Open air
0	97 (6)*	97 (6)	97 (6)	97 (6)	97 (6)	97 (6)	97 (6)
7	96 (3)	94 (6)	97 (3)	86 (11)	13 (14)	83 (7)	93 (3)
14	92 (16)	91 (11)	94 (10)	85 (28)	35 (66)	44 (48)	87 (10)
21	96 (17)	94 (13)	96 (16)	89 (29)	0	0	89 (19)
28	94 (17)	91 (11)	92 (15)	53 (34)	0	0	67 (18)
42	94 (18)	96 (13)	92 (13)	52 (35)	—	—	1 (100)
56	88 (13)	80 (15)	82 (24)	28 (46)	—	—	0
70	87 (16)	81 (21)	85 (28)	5 (20)	—	—	0
84	89 (17)	81 (20)	81 (32)	8 (88)	—	—	—
116	76 (32)	64 (34)	45 (69)	0	—	—	—
148	50 (50)	53 (36)	11 (64)	0	—	—	—
210	36 (56)	37 (41)	5 (100)	—	—	—	—
290	2 (100)	9 (89)	0	—	—	—	—
365	0	1 (100)	0	—	—	—	—
455	0	2 (100)	—	—	—	—	—
555	—	0	—	—	—	—	—

\* Figures in parentheses refer to percentage of weak seedlings.

TABLE II

PERCENTAGE SEED GERMINATION IN *Populus grandidentata* AFTER STORAGE  
AT DIFFERENT RELATIVE HUMIDITIES

Storage time, days	Relative humidity, %						
	7	20	35	52	65	79	Open air
0	98 (6)*	98 (6)	98 (6)	98 (6)	98 (6)	98 (6)	98 (6)
7	81 (7)	97 (10)	97 (5)	91 (10)	91 (11)	92 (11)	90 (9)
14	86 (14)	95 (12)	96 (15)	13 (11)	81 (21)	73 (32)	85 (20)
21	81 (19)	94 (11)	92 (17)	95 (10)	16 (88)	9 (56)	75 (25)
28	79 (29)	86 (15)	87 (20)	89 (14)	0	0	48 (65)
42	48 (42)	82 (13)	74 (27)	50 (36)	0	0	0
56	55 (40)	80 (21)	77 (26)	59 (39)	—	—	0
70	48 (38)	83 (16)	73 (27)	45 (31)	—	—	—
84	53 (42)	62 (23)	62 (29)	44 (48)	—	—	—
116	45 (84)	81 (28)	70 (40)	17 (100)	—	—	—
148	39 (59)	71 (21)	56 (30)	0	—	—	—
210	4 (100)	56 (43)	28 (50)	0	—	—	—
290	0	43 (42)	0	—	—	—	—
365	0	18 (61)	0	—	—	—	—
455	—	9 (78)	—	—	—	—	—
555	—	3 (67)	—	—	—	—	—
731	—	0	—	—	—	—	—

\* Figures in parentheses refer to percentages of weak seedlings.

Moss (4) found relative humidities ranging from 10 to 50% to be about equally effective in maintaining viability (about 13% germination) for 105 days. At 326 days there was no germination in his material.

The good results from seeds stored over calcium chloride (which was included in the study to test a dry desiccant) have found a practical application in long distance shipments of *Populus* seeds. Highly successful exchanges were made with workers in Sweden and Germany by using a sealed glass vial in which the seeds and chloride were separated by a plug of cotton wool. For mailing, the vial was placed in a well-padded medical mailing tube.

### *Ulmus* Experiments

Results in Tables III and IV show that elm seeds retain, after one year's storage, about the same level of viability over a wide range of humidity conditions. The 1940-41 results from seeds of apparently low initial vitality—possibly a seasonal effect—do not clearly indicate an optimum storage humidity. The 1945-46 results, from seeds of higher initial vitality, indicate optimum storage conditions within a range of 20 to 50% relative humidity.

TABLE III

PERCENTAGE SEED GERMINATION IN *Ulmus* AFTER STORAGE AT DIFFERENT RELATIVE HUMIDITIES, 1940-41

Storage time for various species, months	Relative humidity, %				
	7	20	35	52	Open air
<i>U. americana</i>					
0	37	37	37	37	37
6	14	9	18	22	30
12	3	0	11	10	12
Av. of 12 monthly tests	18	13	20	22	20
<i>U. laevis</i>					
0	60	60	60	60	60
6	20	4	25	0	10
12	6	0	8	0	2
Av. of 12 monthly tests	23	19	25	10	15
<i>U. pumila</i>					
0	68	68	68	68	68
6	22	23	20	44	41
12	16	0	8	8	28
Av. of 12 monthly tests	39	20	27	42	47

The data for monthly germination tests are not presented in full in Tables III and IV since the essential facts can be clearly and more briefly indicated by the selected data given.

The stimulatory effect of light on seed germination is shown in Table V. This phenomenon has been reported in other plant species, particularly lettuce (3, pp. 1052, 1053), and is considered to be related to little understood



TABLE IV

PERCENTAGE SEED GERMINATION IN *Ulmus* AFTER STORAGE AT DIFFERENT  
RELATIVE HUMIDITIES, 1945-46

Storage time for various species, months	Relative humidity, %					
	10	20	35	50	65	Open air
<i>U. americana</i>						
0	75	75	75	75	75	75
6	64	71	72	78	63	46
12	59	74	74	74	41	38
Av. of 12 monthly tests	62	73	75	76	60	50
× <i>U. hollandica</i>						
0	82	82	82	82	82	82
6	68	73	79	72	61	58
12	67	76	85	77	60	54
Av. of 12 monthly tests	65	75	81	74	60	58

TABLE V

EFFECT OF LIGHT ON PERCENTAGE SEED GERMINATION IN *Ulmus*

Light condition	<i>U. americana</i>		× <i>U. hollandica</i>		<i>U. laevis</i>		<i>U. pumila</i>	
	1940	1945	1945 (a)	1945 (b)	1940	1945	1940	1945
Fluorescent light	37	63	66	61	60	72	68	65
Total darkness	7	10	13	12	18	20	40	35

photochemical changes within the seed. The pronounced beneficial effect of light on the germination of elm seed as shown in Table V, so far as is known, has not been previously reported.

### Acknowledgments

The author gratefully acknowledges the kind assistance of Mr. M. W. Thistle and Dr. N. H. Grace in the preparation of the manuscript.

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FIG. 1. *Equipment and materials used for the treatment of sprouting seeds.*  
FIG. 2. *Equipment and materials used for the treatment of small seedlings.*

TABLE IV

PERCENTAGE SEED GERMINATION IN *Ulmus* AFTER STORAGE AT DIFFERENT  
RELATIVE HUMIDITIES, 1945-46

Storage time for various species, months	Relative humidity, %					
	10	20	35	50	65	Open air
<i>U. americana</i>						
0	75	75	75	75	75	75
6	64	71	72	78	63	46
12	59	74	74	74	41	38
Av. of 12 monthly tests	62	73	75	76	60	50
× <i>U. hollandica</i>						
0	82	82	82	82	82	82
6	68	73	79	72	61	58
12	67	76	85	77	60	54
Av. of 12 monthly tests	65	75	81	74	60	58

TABLE V

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	1940	1945	1945 (a)	1945 (b)	1940	1945	1940	1945
Fluorescent light	37	63	66	61	60	72	68	65
Total darkness	7	10	13	12	18	20	40	35

photochemical changes within the seed. The pronounced beneficial effect of light on the germination of elm seed as shown in Table V, so far as is known, has not been previously reported.

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## COLCHICINE TREATMENT TECHNIQUES FOR SPROUTED SEEDS AND SEEDLINGS<sup>1</sup>

BY L. P. V. JOHNSON<sup>2</sup> AND H. W. HOLTZ<sup>3</sup>

### Abstract

Colchicine techniques were developed on the principle that sprouted seeds or seedlings should not be completely immersed in colchicine solutions since the root, an earlier developing, more actively dividing, absorptive organ, will become overtreated before the stem receives sufficient treatment. Sprouting seeds are placed, stem end down, in holes in a corrugated rubber disk fitted into a Petri dish containing colchicine solution. A covering of moist filter papers protects the upturned roots from drying. Seedlings are grown in small pots that are inverted over similar pots containing vials of colchicine solution so that the seedlings in the upper pots are immersed in the vials. In both methods a 0.2% aqueous colchicine solution is used, and immersion ranging from 6 to 48 hr. is required depending on the species.

### Introduction

This paper describes methods of colchicine treatment developed at the National Research Council Laboratories over an eight year period of intermittent work directed toward the production of chromosome doubling in plants, particularly forest trees. Most of the published techniques, particularly those outlined by Blakeslee and Avery (1) and by Dermen (2), were tried at one time or another. The methods described below are considered to be the most convenient and effective for inducing chromosome doubling in most plant species commonly propagated by seeds.

### Physiological Considerations

When colchicine is applied to the dividing cell it permits division to go forward to a point where the chromosomes have split, but prevents the normal passage of the two sets of split chromosomes to opposite poles of the cell, which, in turn, prevents the normal division into two cells. Thus, through colchicine treatment the chromosome complements of two cells are retained in one, giving a doubled chromosome number to that cell and to all the cells derived from it.

Since colchicine treatment is dependent upon cell division for its effect, it follows that plant parts that are very actively dividing, such as the root and stem of germinating seeds, should be the objects of treatment.

It was found, however, that when sprouting seeds were completely immersed in colchicine solution, the degree of treatment required to produce the desired effect in the stem invariably produced overtreatment of the root. The reason

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<sup>3</sup> Senior Laboratory Assistant.

for this result seems clear: the root, being an earlier developing, more actively dividing organ is more susceptible to treatment than the stem. This over-treatment, possibly through the production of cells with redoubled chromosome numbers, caused severe physiological disturbances that resulted in distorted stunting and eventual death of the seedlings.

Obviously, the solution to these difficulties was to treat the stem without treating the root. This is the principle upon which the techniques described below are based.

### Techniques

#### *Treatment of Sprouting Seeds*

This method is illustrated by Fig. 1. A disk of a diameter that fits into a Petri dish bottom is cut from corrugated rubber matting. Holes of a diameter determined by the size of the seeds to be used are bored or punched into the grooves of the disk. The disk is placed with corrugations down in a Petri dish containing a shallow depth of 0.2% aqueous colchicine solution. Seeds, previously sprouted on moist blotters, are then placed, stem end down, into the holes of the disk. Moist filter papers are placed over the protruding roots to prevent their drying out and the cover placed on the dish.

The duration of treatment will vary with different kinds of seeds. A convenient series to determine a suitable duration of treatment is 6, 16 (overnight), 24, and 48 hr. Sprouted seeds should be thoroughly washed upon removal from treatment and immediately planted in soil.

#### *Treatment of Small Seedlings*

This method is illustrated by Fig. 2. The seedling to be treated is grown in or transplanted to a 2 or 2½ in. pot and well watered. A second pot of the same size is prepared containing moist sand in which a small shell vial (about 16 × 40 mm.) is embedded. This vial is nearly filled with 0.2% colchicine solution. The first (seedling) pot is then inverted over the second so that the tip of the seedling is immersed in the solution contained in the vial.

A series of trial treatments of 6, 16, 24, and 48 hr. is suggested to determine the duration of treatment suitable for a given kind of seed. The seedling normally requires no attention (e.g., watering) during treatment up to 48 hr. duration, but should be thoroughly rinsed at the conclusion of treatment.

The seedling should be at a very young stage and, if the growing point or leaves have cuticular or other coverings, it is advantageous to scrape them with a sharp instrument.

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# HYBRIDIZATION TECHNIQUE FOR FOREST TREES<sup>1</sup>

By L. P. V. JOHNSON<sup>2</sup> AND E. C. BRADLEY<sup>3</sup>

## Abstract

The relative merits of different materials as coverings to protect receptive female flowers against chance pollination were studied, and a combination of glassine (inner) and kraft (outer) bags was adopted. Pollination was effected as follows: the kraft bag was removed and the glassine bag punctured with the point of a specially designed pollen gun; the pollen was then driven into the bag by squeezing the bulb of the gun; finally, the puncture was sealed with an adhesive patch and the kraft bag replaced. The pollen gun attaches to pollen containers (small Erlenmeyer flasks) that can be stored over calcium chloride.

## Introduction

This paper describes the hybridization technique that has been developed over the past eight years at the National Research Laboratories and the Dominion Forest Service.

When hybridization of forest trees was first undertaken by the senior author in 1938, the methods of Austin (1) and Larsen (4, pp. 93-113) had been published, and were followed later by Schreiner's (5) report on breeding technique. All of these methods were used at one time or another, and certain features of each are combined with some original devices to make up the present method. A previous report (2) described a greenhouse method involving the use of detached branches (suitable for poplar, willow, and elm). The method reported herein is designed for outdoor hybridization work.

## Botanical Considerations

Controlled hybridization requires two conditions: first, that the female flower of the selected female tree be effectively protected from chance pollination during its entire period of receptivity; and second, that the female flower be deliberately and effectively pollinated at the height of its receptivity by pollen of the selected male tree.

The first of these conditions simply requires that a pollen-tight covering (bag) be placed over the unopened (unreceptive) female flower. It is, however, complicated somewhat by the flowering characteristics of certain trees. In dioecious genera (e.g., poplar, willow, certain maples, and certain ashes) male and female flowers occur on separate trees and there need be no concern about self pollen. In monoecious genera (e.g., pine, spruce, fir, larch, birch, oak, walnut, chestnut, beech, alder, and certain maples) male

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and female flowers occur on the same tree, and care must be taken to exclude male flowers when the bag is placed over the female flower. In genera bearing perfect flowers (e.g., basswood, elm, and certain maples) male and female elements are present in the same flower. In this case it will be necessary to remove the male element from the immature flower (emasculation), unless the tree is self-incompatible or possesses a high degree of dichogamy (male and female elements maturing at different times) (3).

### Technique

Pine, a typical monoecious genus, was selected as the demonstrative material for Figs. 1 and 2, which are the bases for the following description of hybridization technique. The equipment and supplies illustrated are those carried in the field during practical hybridization work.

#### *Bagging*

Fig. 1 illustrates the bagging of the unreceptive female flower. The embossed tag, which is the permanent label, is nailed to the tree. After selecting a suitable branch bearing unopened female flowers near the tip, secateurs and scissors are used to trim the needles from a section of the branch axis a few inches from the tip, and to remove any male flowers. A pad of cotton wool is wrapped around the trimmed section and a glassine bag (size 7 × 12 in.) is placed over the branch and tied tightly with a heavy string over the pad. A second pad is wrapped around this tie, and a kraft paper bag (7 lb. size) is placed over the glassine bag and tied so that the pressure is on the pad. Finally, the branch is labelled with a metal tag inscribed with the number of the tree.

The glassine bag is the true protective covering, while the kraft bag is a mechanical protection against wind and rain.

#### *Pollination*

Fig. 2 illustrates the pollination technique. Pollen had previously been collected in a Petri dish or beaker from the tree selected as the male parent, either directly or from detached branches brought into the greenhouse and placed in water a few days before pollen dehiscence. The pollen was then screened and placed in an Erlenmeyer flask, which was loosely plugged with cotton wool and stored until needed in a screw-top jar containing anhydrous calcium chloride under a cotton pad. Pollination is effected by the following steps:

1. Remove the kraft bag.
2. Puncture the glassine bag with the pointed glass tube of the 'pollen gun' and drive a cloud of pollen by squeezing the bulb of the gun two or three times.
3. Seal the puncture with an adhesive patch.
4. Replace kraft bag.
5. Complete inscription on tag by adding designation of the male parent.



FIG. 1. *Equipment and materials used for bagging.*

FIG. 2. *Equipment and materials used for pollination.*





Methanol, which is highly toxic to pollen and evaporates quickly, is used to clean the hands, glassware, pollen gun, etc. in order to prevent contamination that might lead to unwanted male parentage. It is best to have a gun for each kind of pollen. Sufficient pollen may be carried in the flask to pollinate hundreds of female flowers.

### General

The pollen gun design was suggested by the familiar laboratory wash bottle. A close inspection of Fig. 2 will reveal the method of construction. Specifications of materials are as follows: rubber stopper, size 2; glass tubing, size 6 mm.; Erlenmeyer flask, size 50 ml.; rubber bulb, the common atomizer obtainable at drug stores.

Considerable experimentation was carried out to determine the most suitable material for bags. Conclusions were as follows: the 10 oz. army duck bag suggested by Austin (1) is too heavy for the branches of most species; cellophane bags do not permit the necessary passage of moisture transpired by the enclosed branch; parchment bags have great wet strength but, after weathering, become very brittle when dry and break easily; glassine bags, even when used double, do not satisfactorily withstand severe weather; kraft bags are reasonably pollen-tight and withstand rough treatment, except rubbing when wet; the glassine bag covered with a kraft bag gave the best combination of pollen-tightness, strength, and suitable growing conditions for the enclosed branch.

In the manufacture of the glassine bag, the closed end must be so folded that open corners are not possible, and there must be no imperfections in the seam.

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## PRELIMINARY REPORT ON INTERSPECIFIC HYBRIDIZATION IN FOREST TREES<sup>1</sup>

BY L. P. V. JOHNSON<sup>2</sup> AND C. HEIMBURGER<sup>3</sup>

### Abstract

Interspecific hybridization in various forest tree genera gave the following results: *Populus*, 43 crosses; *Picea*, 12; *Pinus*, 4; *Betula*, 14; *Fraxinus*, 5; *Ulmus*, 7; and *Tilia*, 9. The hybridity of most of these materials has been proved or strongly indicated by various criteria; but for some of the materials, hybridity is assumed on the basis of seedling production under conditions that largely precluded the possibility of self- or chance-pollination.

This paper reports the results of interspecific cross pollinations made in various forest tree genera over the period 1938 to 1945, inclusive. The work was done mainly at the Petawawa Forest Experiment Station, Chalk River, and the Dominion Arboretum, Ottawa.

The interspecific crosses in *Populus*, *Picea*, *Pinus*, *Betula*, *Fraxinus*, and *Ulmus*, listed in Tables I to VI, respectively, are those from which seedlings were obtained. For the most part, the hybridity of the seedlings from a cross has been proved, or strongly indicated by various criteria. In some crosses, however, hybridity is assumed on the basis of seedling production under conditions that largely precluded the possibility of self- or chance-pollination.

The crosses in *Tilia* listed in Table VII are considered worthy of report on the basis of the production of apparently sound seeds from cross-pollination of carefully emasculated flowers.

The preliminary nature of this report should be emphasized. A further report in a few years' time would no doubt alter the status of a number of the crosses listed.

Of the numerous cross pollinations that were unsuccessful, only a few were on a sufficiently large scale to make the negative results worthy of report. These, all in *Pinus*, are as follows: *P. resinosa* cross-pollinated with *P. sylvestris*, *P. Banksiana*, and *P. nigra*; *P. sylvestris* cross-pollinated with *P. resinosa* and *P. Banksiana*; and *P. Strobus* cross-pollinated with *P. koraiensis* and *P. resinosa*.

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TABLE I  
 INTERSPECIFIC CROSSES IN *Populus*

Cross	Seed set	Germination, %	Total No. of seedlings	Survival, %	Remarks
<i>P. alba</i> × <i>P. grandidentata</i>	Excellent	61	1559	29	
× <i>P. tremuloides</i>	Excellent	50	92	28	
× <i>P. tremuloides aurea</i>	Good	46	454	12	
× <i>P. canescens</i> (3n)	Poor	29	58	21	
× Northwest poplar	Fair	20	3	3	Doubtful
× ( <i>P. alba</i> × <i>grandidentata</i> )	Fair	21	127	33	
× <i>P. canadensis Eugenei</i>	Good	35	890	0	Definite hybrid
× <i>P. deltoides</i>	Poor	1	9	0	Doubtful
<i>P. alba</i> (3n) × <i>P. grandidentata</i>	Poor	10	1	0	Definite hybrid
<i>P. canescens</i> × <i>P. tremuloides aurea</i>	Excellent	95	5803	85	
× ( <i>P. alba</i> × <i>grandidentata</i> )	—	—	—	—	Nine selected seedlings
<i>P. grandidentata</i> × <i>P. canescens</i> (3n)	Good	84	464	32	
× <i>P. alba</i>	—	—	6	83	
× <i>P. canadensis Eugenei</i>	Fair	28	105	0	Definite hybrid
<i>P. tremuloides</i> × <i>P. tremula</i>	Good	50	103	38	
× <i>P. canescens</i>	Good	38	58	33	
× <i>P. adenopoda</i>	Good	95	196	80	
<i>P. tremuloides aurea</i> × <i>P. adenopoda</i>	Fair	1	2	0	Doubtful
<i>P. (alba</i> × <i>grandidentata</i> ) × <i>P. tremula</i>	Fair	40	51	31	
× ( <i>P. alba</i> × <i>tremuloides</i> )	—	—	—	—	Three selected seedlings
× <i>P. tremuloides aurea</i>	Good	75	1350	75	
× <i>P. canadensis Eugenei</i>	Fair	1	37	70	
× <i>P. canescens</i>	Fair	2	201	40	
<i>P. acuminata</i> × Carolina poplar	Fair	10	65	45	
× Lombardy poplar	Fair	13	50	30	
× <i>P. canadensis Eugenei</i>	Good	20	195	90	
× <i>P. tristis</i>	Fair	Fair	Several	0	Definite hybrid
× <i>P. deltoides</i>	Poor	5	4	25	
× <i>P. Simonii</i>	Fair	50	24	58	
× <i>P. Petrowskyana</i> × ( <i>P. deltoides</i> × <i>Tacamahacca</i> )	Poor	70	75	84	
× <i>P. berolinensis</i> × <i>P. Simonii</i>	Poor	20	4	50	Seven selected seedlings
× <i>P. Tacamahacca</i>	—	—	—	—	Seedlings lost
× Northwest poplar	Good	50	1510	72	
× <i>P. deltoides</i>	Fair	74	675	31	
× Lombardy poplar	Fair	80	520	14	
<i>P. Sargentii</i> × <i>P. Simonii</i>	Good	95	120	80	
<i>P. deltoides</i> × <i>P. Simonii</i>	Poor	2	16	8	
× <i>P. Jackii</i> × Northwest poplar	Fair	41	31	26	
× <i>P. tristis</i>	—	—	—	—	Seven selected seedlings
× <i>P. Rasumowskyana</i> × <i>Tacamahacca</i>	Good	70	494	71	
<i>P. angulata</i> × <i>P. Simonii</i>	—	—	—	—	Nine selected seedlings
<i>P. Tacamahacca</i> × <i>P. Simonii</i>	—	—	—	—	Seedlings lost
× <i>P. tristis</i>	—	—	19	100	

TABLE II  
INTERSPECIFIC CROSSES IN *Picea*

Cross	Seed set, %	Germination, %	Total No. seedlings	Survival, %
<i>P. glauca</i> × <i>P. mariana</i>	47	18	379	73
× <i>P. Abies</i>	39	2	64	56
× <i>P. rubens</i>	41	3	7	61
× <i>P. pungens</i>	30	9	15	52
<i>P. Abies</i> × <i>P. mariana</i>	61	—	25	50
× <i>P. glauca</i>	—	—	36	67
× <i>P. rubens</i>	65	—	—	—
<i>P. rubens</i> × <i>P. mariana</i>	45	2	10	40
× <i>P. Abies</i>	36	5	8	25
<i>P. mariana</i> × <i>P. glauca</i>	28	1	5	80
× <i>P. Abies</i>	54	4	9	33
× <i>P. rubens</i>	—	—	9*	100

\* Pronounced hybrid vigour; all hybrids from the combination, SB-1 × SR-7.

TABLE III  
INTERSPECIFIC CROSSES IN *Pinus*

Cross	Seed set, %	Germination, %	Total No. of seedlings	Survival, %	Remarks
<i>P. Strobus</i> × <i>P. Peuce</i>	55	6	62	70	Growth habit of <i>P. sylvestris</i> dominant Seeds white
<i>P. sylvestris</i> × <i>P. Mugo</i>	22	26	34	83	
× <i>P. nigra austriaca</i>	38	1	3	0	
<i>P. Banksiana</i> × <i>P. contorta latifolia</i>	—	—	50	98	

TABLE IV  
INTERSPECIFIC CROSSES IN *Betula*

Cross	Seed set	Germination	Total No. of seedlings*
<i>B. papyrifera</i> × <i>B. lutea</i> **	Good	Medium-low	144
× <i>B. populifolia</i>	Good	Medium	135
× <i>B. lenta</i>	Fair	Medium	351
× <i>B. nigra</i>	Fair	Low	48
× <i>B. pubescens urticifolia</i>	Good	Low	156
<i>B. macrophylla</i> *** × <i>B. lutea</i> **	Fair	Medium-low	39
× <i>B. papyrifera</i>	Good	Medium	480
× <i>B. nigra</i>	Fair	Low	137
× <i>B. pubescens urticifolia</i>	—	Medium-low	312
<i>B. populifolia</i> × <i>B. papyrifera</i>	Good	Low	193
× <i>B. lenta</i>	Good	Low	57
<i>B. lenta</i> × <i>B. papyrifera</i>	Fair	Low	28
× <i>B. lutea</i>	Fair	Medium	479
<i>B. pubescens</i> × <i>B. papyrifera</i>	—	—	30

\* Survival good in all crosses.

\*\* Wintergreen flavour in the hybrid.

\*\*\* Not described by Rehder (6).

TABLE V  
INTERSPECIFIC CROSSES IN *Fraxinus*

Cross	Seed set, %	Germination, %	Total No. of seedlings*
<i>F. excelsior</i> aureo-variegata × <i>F. americana</i>	60	28	570
× <i>F. pennsylvanica</i> **	75	34	602
× <i>F. quadrangulata</i> ***	40	20	193
<i>F. Richardi</i> † × <i>F. americana</i>	50	19	461
× <i>F. pennsylvanica</i>	1	10	2

\* Survival good in all crosses.

\*\* Very variable; hybrid vigour.

\*\*\* Hybrid vigour.

† Not described by Rehder (6).

TABLE VI  
INTERSPECIFIC CROSSES IN *Ulmus*

Cross	Seed set, %	Germination, %	Total No. of seedlings*
<i>U. fulva</i> × <i>U. americana</i>	20	7	50
<i>U. hollandica</i> superba × <i>U. pumila</i>	60	46	549
<i>U. laevis</i> × <i>U. americana</i>	70	35	624
× <i>U. Thomsii</i>	18	10	37
<i>U. procera</i> × <i>U. pumila</i>	50	13	132
<i>U. pumila</i> × <i>U. fulva</i>	60	41	205
× <i>U. hollandica</i> major	60	11	116

\* Survival good in all crosses.

TABLE VII  
INTERSPECIFIC CROSSES IN *Tilia*

Cross	Seed set, %	No. of apparently sound seeds*
<i>T. americana</i> × <i>T. platyphyllos</i> rubra	22	84
× <i>T. platyphyllos</i> vitifolia	2	27
× <i>T. europaea</i> pallida	12	34
× <i>T. euchlora</i>	60	12
× <i>T. Moltkei</i>	50	6
<i>T. americana</i> macrophylla × <i>T. platyphyllos</i> rubra	50	12
<i>T. Moltkei</i> × <i>T. americana</i>	25	6
× <i>T. platyphyllos</i> rubra	50	12
× <i>T. platyphyllos</i> vitifolia	90	12

\* Seeds accidentally destroyed.

Results of the earlier hybridization work in *Populus* have already been reported (1, 2). Details of the hybridization methods used in the work generally have also been published (3, 4, 5).

The nomenclature of Rehder (6) has been used throughout the paper.

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## A NOTE ON INHERITANCE IN $F_1$ AND $F_2$ HYBRIDS OF *POPULUS ALBA* L. $\times$ *P. GRANDIDENTATA* MICHX.<sup>1</sup>

BY L. P. V. JOHNSON<sup>2</sup>

### Abstract

At five years of age, average heights in feet were: *Populus alba*, 14.0; *P. grandidentata*, 11.7;  $F_1$ , 17.6;  $F_2$ , 9.5. On the basis of an  $F_2$  population of 168 trees, modes of inheritance were assumed for leaf characters as follows: pubescence, four cumulative factors; colour, 1 : 2 : 1 monohybrid ratio; midrib, 42 : 15 : 6 : 1 trihybrid ratio; shape, margin, apex, and base, each inherited on the basis of multiple factors.

The high rooting capacity of *P. alba* (92%) was transmitted to the  $F_1$  hybrid as a dominant character. It is suggested that *P. alba* is homozygous for one or more highly-determining, dominant alleles not possessed by *P. grandidentata*. Backcrossing failed to increase the degree of rooting. Comparable results from *P. tremuloides* indicated that this species carries an inhibitor for the *P. alba* rooting factor or factors and is deficient for the rooting factor or factors possessed by *P. grandidentata*.

### Introduction

Information on inheritance in forest trees is almost entirely limited to comparisons between parental and  $F_1$  materials (5, 9), with only a few reported observations on the  $F_2$  having come to the author's notice, as follows: in chestnut (2, 3, 11 (pp. 1242-1279)), larch (7 (pp. 69-222)), oak (12), pine (1), spruce (7 (pp. 69-222)), and willow (4, 10\*). For the most part these observations on the  $F_2$  are of an incidental nature, only two of the reports (4, 7 (pp. 69-222)) purporting to provide information on modes of inheritance. As a matter of fact, the mode of inheritance may be only assumed from  $F_2$  data, the  $F_3$  being required to prove (or disprove) the assumption.

This lack of information may be attributed mainly to two facts: first, the long period between generations represents a time obstacle to genetic studies; and second, the great emphasis being placed on direct utilization of the  $F_1$  diverts attention from the  $F_2$  and, indeed, tends to obviate the practical need for genetic information that might be derived from this and higher generations. There are instances, however, when the fortuitous characteristics of the  $F_1$  are undesirable. Then the breeder must resort to selection among  $F_2$  segregates and, if vegetative propagation is not feasible, must make a genetic study of the material in order to assure the selection of suitable, true-breeding types with a minimum of effort.

<sup>1</sup> Manuscript received July 30, 1946.

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1463.

<sup>2</sup> Formerly Geneticist, National Research Laboratories, Ottawa; now Associate Professor of Agronomy, University of Florida, Gainesville, Fla.

\* Quotation on p. 180 from Wichura, M. *Die Bastardbefruchtung in Pflanzenreich, erläutert an den Bastarden der Weiden*. Breslau, 1865.



The information contained in the present paper may be considered as a small beginning in the building up of a body of genetic information in *Populus* that will become increasingly useful in the breeding work of the genus.

## Materials and Methods

### *Morphological Studies*

A natural cross between a female *Populus alba* L. (A-4), growing near Ottawa, and *P. grandidentata* Michx. (possibly G-11) occurred some years ago, giving sexually mature  $F_1$  individuals of both sexes. In March, 1940, controlled crossing (6) of male ( $A \times G-37$ ) and female ( $A \times G-33$ )  $F_1$  trees produced 1840  $F_2$  seedlings. Mortality among these seedlings, mainly due to damping-off, reduced their numbers so that after one year in the nursery only 195 trees remained.

In May, 1941, the  $F_2$  seedlings, together with parental and  $F_1$  clones derived from cuttings rooted in May, 1940, were transplanted to a gently sloping, cultivated area having a uniform loam soil. The spacing was  $7 \times 7$  ft. in a triangular plan. Subsequent mortality, mainly due to mechanical injury, reduced the  $F_2$  population to 168 trees.

The data in Table I were compiled from observations made in August, 1945, and from leaf specimens collected at that time and preserved in a plant press for detailed study during the following winter.

### *Rooting Studies*

*P. alba* -36 represents a particularly high-rooting individual of this introduced species; all other parental materials are native, unselected forms. The  $F_1$  and backcross materials were selected from fairly extensive populations on the basis of superior rate and type of top growth, without prior knowledge of rooting capacity.

For the rooting tests, 6-in. cuttings were made from current-season wood and heeled in under moist sand in a frost-free chamber. In mid-January the cuttings were planted in fairly coarse builder's sand in a greenhouse bench covered with a muslin screen. Greenhouse temperature was approximately 70° F. Each kind of material was tested in three replicates of 25 cuttings each.

The data given in Table III are from observations made 43 days after planting.

## Results and Discussion

Attention is particularly drawn to the fact that the modes of inheritance outlined in Table I are merely assumed on the basis of the data presented.

Fig. 1 illustrates some of the leaf characters described in Table I. Both upper and lower leaf surfaces are shown for the parental species and the  $F_1$ . The leaves (lower surface only) from  $F_2$  trees were selected to indicate the range of variability for the various characters. There is no numerical significance to the selection of types.

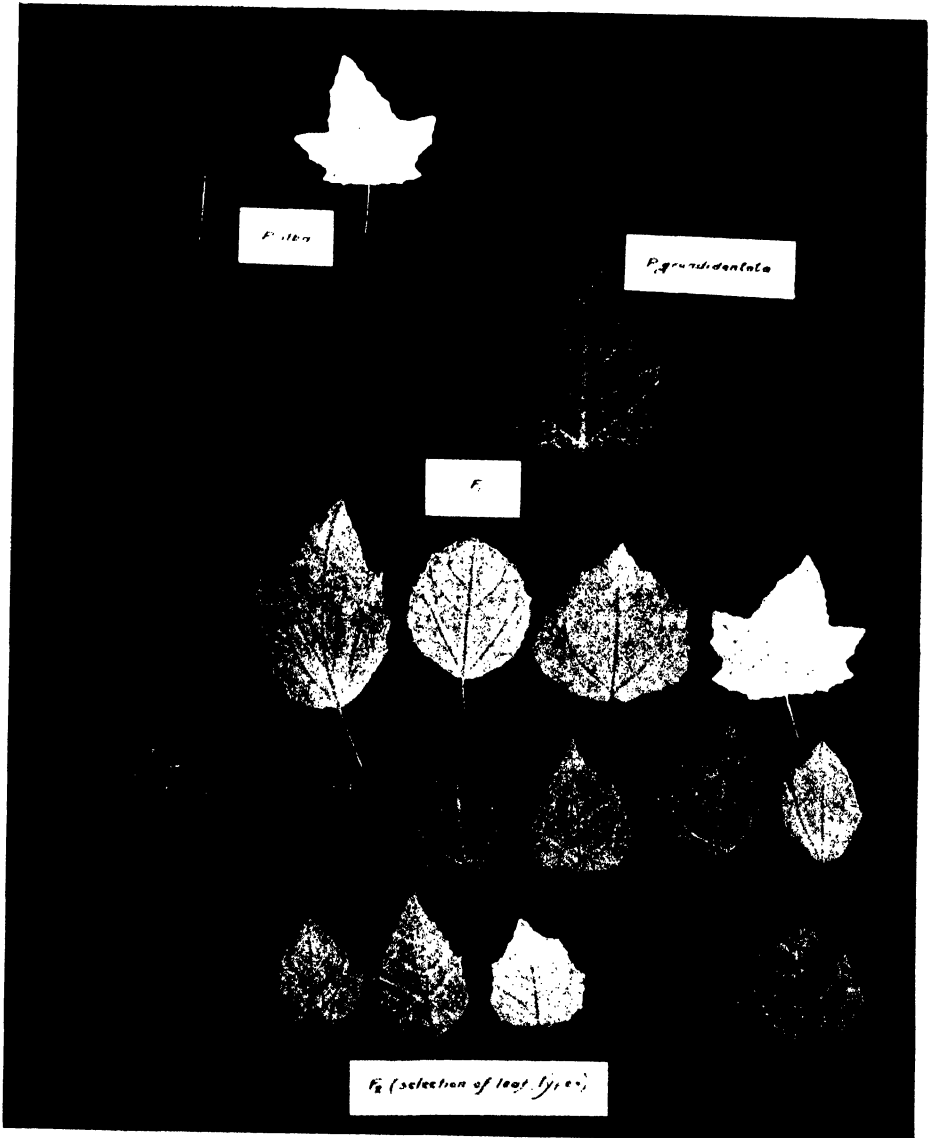


FIG. 1. Leaf types of parents,  $F_1$ , and selected  $F_2$  segregates of the cross, *P. alba*  $\times$  *P. grandidentata*.



TABLE I  
DATA ON LEAF CHARACTERS AND HEIGHT OF THE PARENTS,  $F_1$  AND  $F_2$  OF THE CROSS, *P. alba* (A)  $\times$  *P. grandidentata* (G),  
WITH SUGGESTED MODES OF INHERITANCE

Character	A-4, clone of 3 trees	G-11, clone of 3 trees	A-4 $\times$ G-0, $F_1$ , clone of 3 trees for each sex*	$F_2$ population, 168 trees	Assumed mode of inheritance
Average height, ft.	14.0	11.7	17.5, female 17.7, male	9.5	Multiple factors; loss of heterosis (see Table II)
Leaf: Shape	Triangularly palmate**	Ovate	Deltoid-ovate, sublobulate (intermediate)	Ovate; deltoid; cordate; various degrees of lobing	Multiple factors; variety of shapes too complex to classify; tendency for non-lobed to be dominant (see Fig. 1)
Pubescence, under side	White tomentose	Glabrescent	Grey pubescent	White tomentose to sparsely pubescent (one A type but no G type recovered)	Four cumulative factor pairs (each parental type expected once in 256)
Colour, upper side	Dark green	Light green	Medium green	30 dark green, 94 medium green, 44 light green	Conforms to a 1:2:1 monohybrid ratio
Midrib	Grooved	Non-grooved	Non-grooved	106 non-grooved, 41 slightly grooved, 18 semi-grooved, 3 grooved	Conforms to 3-factor ratio, 42:15:6:1 (3 or more dom. alleles give non-grooved, 2 give sl. grooved, 1 gives semi-grooved, 0 gives grooved)
Margin	Coarsely, irregularly dentate	Coarsely, sinuate-dentate	Intermediate	Coarsely to finely toothed; regular to irregular	Multiple factors; variety of types too complex to classify
Apex	Acuminate	Acuminate	Acuminate	Long-pointed to rounded	Multiple factors; variety of types too complex to classify
Base	Truncate or slightly rounded	Truncate to broad cuneate	Truncate, rounded to subcordate	Truncate; rounded; cuneate; cordate; blended types	Multiple factors; variety of types too complex to classify

\* G-0 denotes open pollination by *P. grandidentata*; G-0 and G-11 may be identical.

\*\* The terminology of Rehder (8) has been followed throughout.

In Table II it is important to note the relatively low vigour of a preponderance of the  $F_2$  segregates. If, because of an unfavourable combination of characters in the  $F_1$ , it became necessary to grow the  $F_2$ , such loss of vigour would seriously reduce the prospects of combining growth vigour with homozygosity and other essential characteristics. Previous reports (1, 2, 7 (pp. 69-222)) have indicated marked reduction of vigour in the  $F_2$  as compared to the  $F_1$ .

TABLE II  
FREQUENCY DISTRIBUTION OF  $F_2$  TREES IN VARIOUS HEIGHT CLASSES

Height classes, ft.								
1.1-3.0	3.1-5.0	5.1-7.0	7.1-9.0	9.1-11.0	11.1-13.0*	13.1-15.0**	15.1-17.0	17.1-19.0***
6	11	25	27	28	30	27	8	6

\* *P. grandidentata* height class.

\*\* *P. alba* height class.

\*\*\*  $F_1$  height class.

TABLE III  
ROOTING FROM CUTTINGS OF *P. alba* (A), *P. grandidentata* (G), *P. tremuloides* (T),  
AND CERTAIN HYBRIDS BETWEEN THESE SPECIES

Expt. No.	Material	Average no. of rootlets per rooted cutting	Rooting	
			Average, %	Means of transformed data*
1	A-36	6.3	92.0	73.9
2	G-8	1.0	5.3	10.6
3	A-36 × G-8 -45	2.7	65.3	54.0
4	-46	5.0	97.7	76.9
5	-47	2.3	92.0	74.5
6	-48	2.0	84.0	66.4
7	-49	2.7	76.0	60.9
8	-50	1.7	76.0	61.2
9	A-36 × G-0**	2.7	68.0	55.8
10	A-17 × G-0**	2.7	60.0	50.9
11	A-36 × (A-17 × G-0) - 9	2.0	78.7	62.9
12	-10	2.3	70.7	57.6
13	T-13	—	0.0	0.0
14	A-36 × T-13	1.3	9.3	17.7
15	T-14	—	0.0	0.0
16	A-36 × T-14	—	0.0	0.0

\* Necessary difference: 5% level of significance, 9.3; 1% level, 12.5. Transformed by:  $\theta = \sin^{-1} \sqrt{p}$ , where  $p$  is the observed proportion, according to the method of Fisher and Yates (Fisher, R. A. and Yates, F. Statistical tables for biological, agricultural and medical research. 2nd ed. Oliver & Boyd, Ltd., London. 1943.).

\*\* Open pollination by *P. grandidentata*.

The rooting data in Table III show no significant difference (5% level) between *A*-36 and  $F_1$  hybrids Nos. 46, 47, and 48; and the other hybrids of this group (Expt. Nos. 3, 7, 8, and 9) definitely fall into the *A*-36 rooting class. These facts strongly suggest that the high rooting capacity of *A*-36 is transmitted to the hybrid as a dominant character. It appears probable that *A*-36 is homozygous for one or more highly-determining, dominant alleles not possessed by *P. grandidentata*.

The results from the backcross (Expt Nos. 11 and 12) support the assumption of dominant *A*-36 rooting factor or factors, since the combining of dominant alleles from *A*-36 and the  $F_1$  does not give rooting above the normal level for the  $F_1$ .

The numbers of rootlets on hybrid cuttings tend to be intermediate between those of the parents.

The results from *P. tremuloides* Michx. indicate that this species is probably deficient for the rooting factor or factors possessed by *P. grandidentata*, and further that it carries an inhibitor that is highly effective against the *A*-36 rooting factor or factors.

### Acknowledgments

The author gratefully acknowledges the assistance of Messrs. Alan Tennant, H. W. Holtz, and E. C. Bradley during the course of the work, and of Mr. M. W. Thistle and Dr. J. M. Armstrong in writing the manuscript.

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## THE EFFECT OF CHANGES OF AIR PRESSURE UPON THE GERMINATION OF CONIDIA OF BARLEY POWDERY MILDEW<sup>1</sup>

BY HAROLD J. BRODIE<sup>2</sup> AND J. F. JONES<sup>3</sup>

### Abstract

Reduction of the pressure by not more than 200 mm. of mercury, of the air surrounding the conidia of *Erysiphe graminis Hordei* Marchal, in water-saturated atmosphere at 18° C., caused an increase in germination percentage when the experimentally treated conidia were compared with conidia allowed to germinate at atmospheric pressure for three hours. At pressure reduction between 200 and 500 mm., germination was only 90% of germination at atmospheric pressure. At pressure reduction more than 500 mm. below atmospheric, germination was impaired although 53% germination was observed at an actual pressure of only 92 mm., and 5 to 6% at 30 to 50 mm. The enhancement of germination under slightly reduced pressure was found to result from an acceleration of the germination process. More conidia in a given sample germinated at reduced pressure within three hours than at atmospheric, but after five hours the germination percentage tended to become equal in the treated and untreated spores. It is suggested that a slight reduction in pressure might allow carbon dioxide to escape more rapidly from the protoplast of a conidium freshly dislodged from its parent conidiophore thus making it possible for germination to proceed more rapidly than in conidia at atmospheric pressure. Increase in pressure was found to depress germinability, but sufficient data were not obtained to determine the exact relationship between pressure increase and germinability. The data recorded offer further confirmation of the previous work by the senior author on the subject.

### Introduction

During the course of investigations concerning the germination of the conidia of the Erysiphaceae (1, 2, 3), the senior author observed that air pressure appeared to have some effect upon the extent to which a sample of conidia under observation would germinate. This was noted particularly in certain experiments involving the use of glass apparatus constructed for the study of the effect of various gases, such as carbon dioxide and nitrogen, upon germination. In a closed system, the mere forcing into place of a stopper, resulting in an increase in air pressure within the system, appeared to interfere with germination. This conclusion was reached after other explanations had been investigated. A rough test was made to determine whether reduction of air pressure might also affect germination and this experiment showed that germination was actually stimulated by slight reduction below the atmospheric pressure obtaining at the time of the experiment.

These observations appeared to have significance in connection with the theory advanced by Brodie and Neufeld (3) to account for the following facts. The conidia of many of the Erysiphaceae are able to germinate readily in dry air (2). To instigate the germination of such spores, it is necessary

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merely to dislodge them from their parent conidiophores by shaking them on to a dry glass slide. Brodie and Neufeld stated that since (in dry air) germination in these fungi is not initiated by the spores absorbing water, some other explanation should be sought for the observation that powdery mildew conidia remain dormant *in situ* and begin to germinate immediately after being abstricted from the parent conidiophore even in dry air. According to these authors, the conidia, while still attached to the parent conidiophores, are in a relatively static condition with regard to the oxygen and carbon dioxide content of the protoplasts. Since it was shown that germination is checked by an atmosphere containing 10% or more of carbon dioxide, it was suggested that the escape of that gas from the protoplast is essential for germination. This gaseous exchange cannot take place to a sufficient extent while the conidia are attached but begins as soon as they have been dislodged. The exchange is believed to take place chiefly through the papilla, which has become exposed to the air, the papilla being regarded as a special structure having greater permeability than the rest of the conidium wall (2, 3).

If the ideas set forth above are correct, then one might expect that changes in air pressure would have an effect upon germination, and the observations of the senior author that germination does appear to be affected by such changes were considered sufficiently interesting to warrant further study.

### Materials and Methods

Barley mildew (*Erysiphe graminis Hordei*) Physiologic Race 8 was used throughout the present work. The fungus was allowed to develop on barley plants (variety O.A.C. 21) that were kept in a large glass case in the laboratory. Host plants were grown under continuous light to prevent the physiologic resistance to mildew that the plants develop when grown in the diffuse light of the laboratory (6). A 200-watt electric light bulb, set in a white reflector and suspended one foot above the glass case, provided satisfactory illumination.

In collecting spore samples, six clean glass microscope slides were placed at the bottom of a tall bell jar and heavily infected leaves were shaken over the bell jar to allow dislodged conidia to settle upon the slides. By this means, fairly uniformly mixed spore samples were obtained. Usually three of these samples were subjected to experiment and three kept as control.

Approximately a thousand conidia were counted for each germination percentage recorded. In examining the spore laden slides, dense fields were avoided when possible because of the difficulty of counting accurately. To avoid any inconsistency caused by possible variation in spore material from day to day, germination data are recorded as per cent of control. Because an hour was required to count conidia for each experiment, it became necessary to guard against the possibility that spores ungerminated at the beginning of the counting period might have germinated during that time. At the conclusion of an experiment, therefore, spores were fixed by exposing them to the fumes of glacial acetic acid in a covered dish, which treatment prevented further germination but did not cause any germ tubes to shrivel.



The apparatus employed for the germination of conidia at various degrees of reduced pressure has been described by the junior author (5) and need be dealt with only briefly here. Two heavy glass germination chambers (Fig. 1, C, D) were immersed in a water-bath supplied with cold running water and maintained as closely as possible at 18° C., this being the lowest temperature near the optimum for germination (12° to 15° C.) that could be maintained in the absence of an immersible refrigerating unit. By using cold running

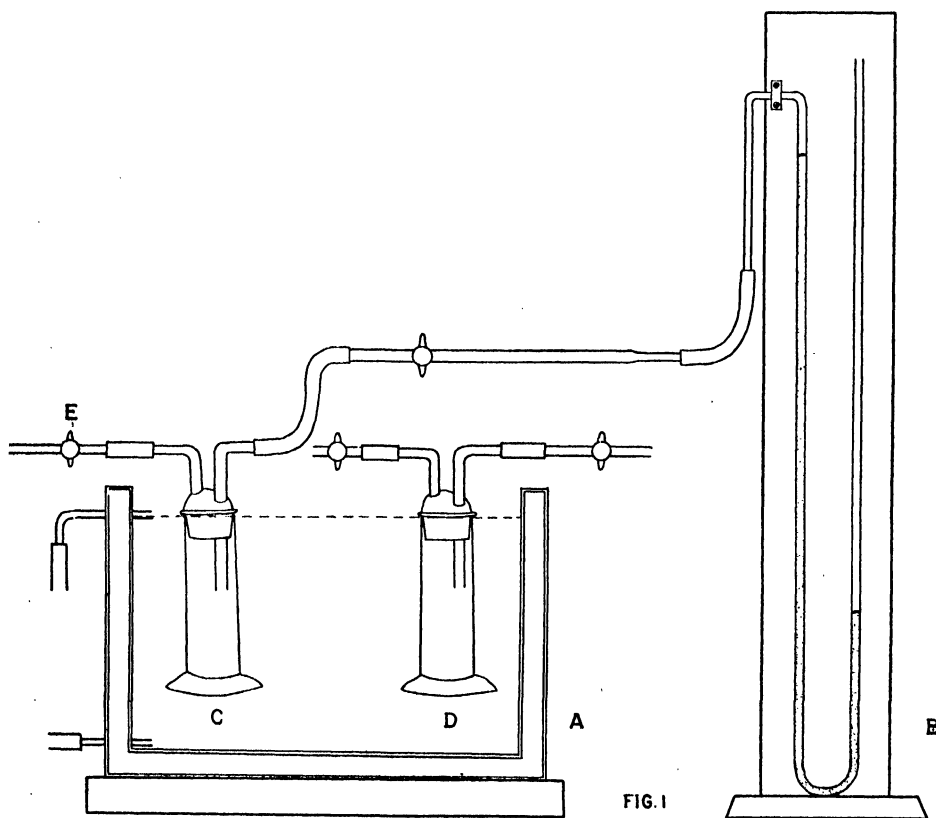


FIG. 1. Diagram of apparatus used in experiments on effect of air pressure: A, water-bath; B, open mercury manometer; C, germination chamber in which air pressure was reduced or increased; D, control germination chamber; E, stopcock.  $\times 1/5$ .

water and ice and maintaining constant vigilance during the experiment, the temperature was kept at 18° C. When the temperature of the laboratory was high a slight variation of  $\pm 1^\circ$  could not always be avoided, but it is felt that such variations did not affect the experimental results since the germination behaviour of any spore sample was always recorded as per cent of the control at precisely the same temperature.

The experiment chamber (Fig. 1, C) was connected on the one side to an open manometer (Fig. 1, B) and on the other, through a trap to a powerful

water aspirator capable of reducing the pressure to about 35 mm., a rarefaction sufficient for the work. The germination chamber is shown in detail in Fig. 2, with the spore laden slides in place. A disk of moist blotting paper, placed at the bottom of each germination chamber insured a moisture-saturated atmosphere comparable in all experiments. The pressure in the germination

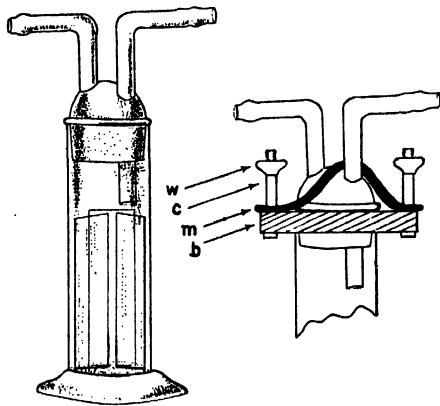


FIG. 2

FIG. 3

FIG. 2. Germination chamber showing spore laden slides in place.  $\times 1/3$ .

FIG. 3. Clamp holding stopper in place when air pressure in germination chamber was above atmospheric: b, wood block base; m, metal clamp; c, collar; w, wing nut.  $\times 1/3$ .

chamber (Fig. 1, C) was reduced as rapidly as possible, the glass taps closed, and conidia allowed to germinate at reduced pressure (in C, Fig. 1) and at atmospheric pressure (in D, Fig. 1) at the same temperature and under the same light conditions. At the end of the experiment and before the spores were removed from the apparatus for counting, the tap nearest the manometer was opened to determine whether any leakage of air into the germination chamber had occurred. The apparatus was considered sufficiently air-tight when it was observed that a drop of not more than a millimeter or two was the most that occurred at the lowest pressures involved and no detectable change at higher pressures.

The apparatus used for germinating conidia under increased air pressure was similar to that described above, the germination chamber being connected to an air pump instead of to an aspirator. Because the pressure tended to force out the vaselined ground glass stopper of the pressure chamber, the latter was held in place by means of a specially constructed clamp (Fig. 3).

In the study of the effect of reduced air pressure upon the time required for germination, two identical pieces of apparatus, shown in Fig. 4, were employed, each consisting of a length of glass tubing sealed with Canada balsam on the lower side to a glass microscope slide (Fig. 4, c) and provided with two outlet tubes, one equipped with a stopcock. These tubes connected the germination

chamber respectively to an aspirator and to a manometer or, in the control chamber, terminated in taps that were closed during the experiment. The microscope slide (Fig. 4, *b*) bearing conidia was inverted over the chamber and sealed on by means of wax, which provided a sufficiently air-tight seal as was indicated by the constancy of the level of mercury in the manometer.

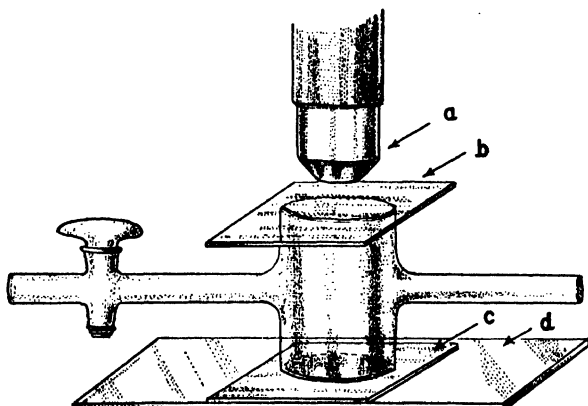


FIG. 4

FIG. 4. Apparatus for observing germination of spores under reduced pressure: *a*, objective of microscope; *b*, glass slide bearing spores on lower side; *c*, glass slide cemented to chamber; *d*, glass slide base for apparatus.  $\times 7/10$ .

Conidia on the under surface of both experiment and control chamber were observed with microscopes both of which derived their light from a common light source, the illumination in each being adjusted as nearly as possible by eye to be of equal intensity. Owing to the impracticability of immersing the apparatus in a constant temperature bath, the temperature during each experiment was recorded.

Great differences in germinability of spores were encountered from experiment to experiment. When the germination percentage recorded in the control was too low to allow for significant comparison with experimentally treated spores, the results were discarded. In any case, as mentioned above, germination in the experiment was expressed as per cent of control to overcome the effect this variation might have on results.

Experience having shown that germination of conidia of *Erysiphe graminis* (as for other mildews previously studied) proceeds most successfully in laboratory experiments, especially during the dull months of early winter, when conidia are removed from the host plants at about 11 a.m.; this practice was adopted throughout the course of the present work.\*

\* The mid-day optimum germinability would appear to be caused by the stimulatory effect of light upon germination, particularly at temperatures below 25° C., which has been observed by Yarwood (7), Cherewick (4), and the junior author (5).

## EXPERIMENTAL DATA

A—EFFECT OF REDUCTION OF AIR PRESSURE UPON  
GERMINATION OF CONIDIA

TABLE I

EFFECT OF REDUCTION OF AIR PRESSURE UPON GERMINATION OF CONIDIA  
AT 18° C. ( $\pm 1^\circ$ ); JULY 1944

Reduction in air pressure in mm. mercury	Germination as per cent of control	Reduction in air pressure in mm. mercury	Germination as per cent of control
44	102	300	88
96	103	400	94
105	106	405	85
172	119	510	76
172	111	550	77
173	117	650	43
175	117	693	5
215	109	695	0
250	93		

TABLE II

EFFECT OF REDUCTION OF AIR PRESSURE UPON GERMINATION OF CONIDIA  
AT 18° C. ( $\pm 1^\circ$ ); JAN., FEB., 1945

Reduction in air pressure in mm. mercury	Germination as per cent of control	Reduction in air pressure in mm. mercury	Germination as per cent of control
31	104	391	95
58	113	488	91
59	109	549	75
91	117	640	62
122	116	658	53
234	93	710	6
235	89	712	0

TABLE III

EFFECT OF REDUCTION OF AIR PRESSURE UPON GERMINATION OF CONIDIA AT 18° C.  
( $\pm 1^\circ$ ). AVERAGE OF ALL DATA OBTAINED 1942 TO 1945

Reduction in air pressure in mm. mercury	Germination as per cent of control	Reduction in air pressure in mm. mercury	Germination as per cent of control
0 - 49	105	400 - 449	89
50 - 99	108	450 - 499	91
100 - 149	107	500 - 549	76
150 - 199	102	550 - 599	76
200 - 249	98	600 - 649	62
250 - 299	91	650 - 699	51
300 - 349	92	700 - 740	3
350 - 399	89		

## B—EFFECT OF INCREASE IN AIR PRESSURE UPON GERMINATION

TABLE IV

EFFECT OF INCREASE IN AIR PRESSURE UPON GERMINATION OF CONIDIA  
AT 18° C. ( $\pm 1^\circ$ ); MARCH 1945

Increase in pressure in mm. mercury	Germination as per cent of control	Increase in pressure in mm. mercury	Germination as per cent of control
40	88	415	57
84	90	493	75
101	97	516	92
200	100	527	70
300	78	576	100
374	64	624	90
377	70	665	73

C—COMPARISON OF TIME REQUIRED FOR GERMINATION OF CONIDIA UNDER  
CONDITIONS OF REDUCED AND ATMOSPHERIC PRESSURE RESPECTIVELY

TABLE V

EFFECT OF REDUCTION OF AIR PRESSURE ( $-125$  MM.) UPON TIME REQUIRED FOR GERMINATION  
OF CONIDIA AT ROOM TEMPERATURE

Expt. No.	Temperature	Time in minutes	Germination, %	
			At reduced pressure	At atmospheric pressure
4	20° C.	30	1	1
		45	7	4
		60	10	6
		75	12	7
		90	14	8
		105	14	9
		120	17	10
		135	18	11
		150	19	12
		165	19	12
		210	20	17
		225	20	19
7	20° C.	75	12	5
		90	18	8
		105	22	10
		120	25	12
		135	27	14
		150	29	15
8	21° C.	30	2	2
		60	7	5
		90	12	7
		120	18	10
		150	22	13
		180	24	15
		210	26	17
		285	26	19

TABLE V

EFFECT OF REDUCTION OF AIR PRESSURE (—125 MM.) UPON TIME REQUIRED FOR GERMINATION OF CONIDIA AT ROOM TEMPERATURE—*Concluded*

Expt. No.	Temperature	Time in minutes	Germination, %	
			At reduced pressure	At atmospheric pressure
11	20° C.	30	1	0
		45	3	1
		75	10	6
		105	17	10
		135	20	12
		195	22	14
		220	24	15
12	22° C.	30	4	3
		45	6	5
		90	15	9
		180	23	16
		225	25	17
		300	27	21
		315	27	26

TABLE VI

EFFECT OF REDUCTION OF AIR PRESSURE (—125 MM.) UPON TIME REQUIRED FOR GERMINATION OF CONIDIA AT 8° C.\*

Expt. No.	Time in minutes	Germination, %	
		At reduced pressure	At atmospheric pressure
15	45	2	0
	60	3	1
	75	4	2
	90	5	2
	105	6	3
	180	7	4
	310	9	8
16	45	1	0
	90	6	8
	105	6	4
	135	9	8
	195	10	9
	225	11	10

\* Experiments conducted in an unheated room at 8° C.

### Discussion of Results

#### A—EFFECT OF REDUCTION OF AIR PRESSURE UPON GERMINATION

The data presented in Tables I, II, and III, which are also shown on graphs in Fig. 5, appear to indicate: (1) that a reduction in pressure not greater than 200 mm. of mercury below atmospheric pressure produces an enhancement of

germination of the conidia of *Erysiphe graminis Hordei*, which enhancement reaches its maximum value at a pressure reduction of about 125 mm.; (2) that pressure reduction between 250 mm. and 500 mm. below atmospheric level depresses germination to about 90% of control; and (3) that pressure reduction between 500 mm. and 720 mm. causes a marked depression in germination, which is closely related to the pressure reduction.

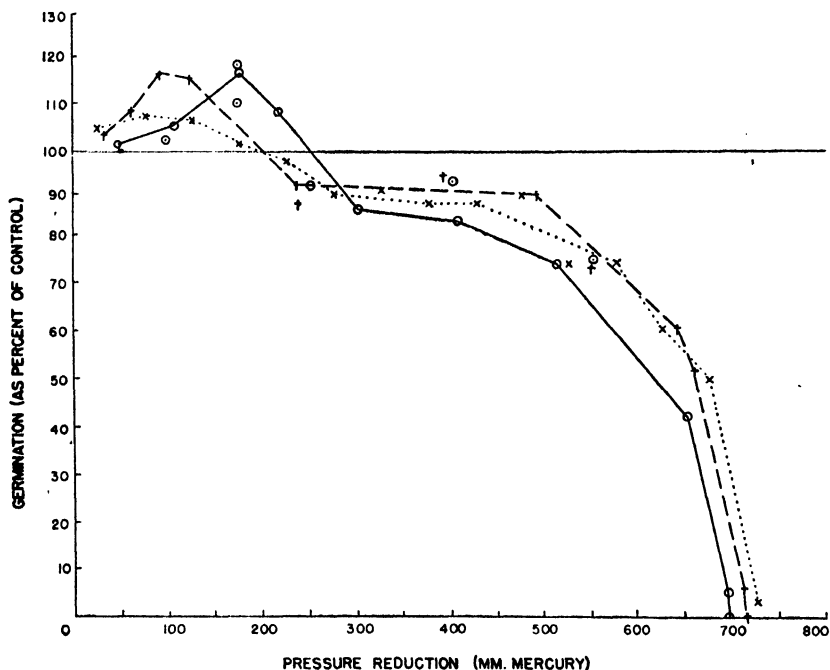


FIG. 5. Graph showing effect of reduction of air pressure upon germination of conidia of *Erysiphe graminis Hordei* at 18° C. ○ = Table I; † = Table II; × = Table III.

It is worthy of note that although the data presented in Table I were obtained six months before the data of Table II, the similarity in character of the graphs (Fig. 5) is considerable. The graph (Fig. 5, Table III) derived from Table III is included for comparison and represents the averages of all data obtained including data not otherwise reported in this paper. While such a graph may have doubtful value statistically, it may be noted that it does show the same general characters as the two graphs derived respectively from Tables I and II.

Realizing that the experiments on pressure reduction are limited in number because of the time-consuming nature of the work, and acknowledging that an explanation other than the one suggested is possible, the following comments are offered regarding the behaviour of the mildew conidia when subjected to reduced pressure.

Examining the graphs shown in Fig. 5 it will be noted that their courses appear to fall into three stages:

(1) The region from 0 to about 200 mm. pressure reduction. Over this pressure range, germination is stimulated. In line with the senior author's previously stated hypothesis (1, 3), which postulates that, for germination to take place, carbon dioxide must escape from the conidium and oxygen enter, it seems possible that in some way a slight rarefaction of the air surrounding the conidia might facilitate the escape of carbon dioxide from the protoplast, thus allowing a higher percentage of spores to germinate in a given time than are able to germinate at atmospheric pressure.

(2) The region from 200 to 500 mm. pressure reduction. Over this second pressure range, germination at reduced pressure remains more or less constant in the neighbourhood of 90% of germination at atmospheric pressure.

Beyond 200 mm. reduction, the stimulatory effect observed at lower values is lost. The stimulation effected by the initial slight pressure reduction might not be manifest because the pressure is rapidly reduced still further (the time required for rarefaction being between 30 sec. and one minute). Further, the *total amount* of oxygen in the closed system is reduced when air is pumped out. This may very well cause the slight depression of germination observed and if so, one is led to assume that, within this region of the curves, the amount of oxygen removed has not passed a critical value, a suggestion that finds support in the rate at which germination falls off beyond a pressure reduction of 500 mm.

(3) The region from 500 to 720 mm. pressure reduction. Germination falls rapidly and regularly. Pressure reduction of 697 mm. and 712 mm. were required in the 1944 and 1945 experiments, respectively, to cause germination to cease entirely, and it is remarkable that conidia were able to germinate to the extent of 5 or 6% of the spores examined, in a rarefied atmosphere having not more than approximately 30 to 50 mm. pressure.\* The most obvious explanation for the decline in germination over this region of the curves would appear to be the gradual withdrawal of air within the closed system, the total amount of oxygen available for life being reduced.

#### B—EFFECT OF INCREASE IN AIR PRESSURE UPON GERMINATION

When plotted (graphs not included in this paper), the data presented in Table IV display no clear-cut regular arrangement. On the whole, they appear to show that increase in air pressure results in a depression of germination. Whether there is some regularity in the extent to which germination is hindered by increase in air pressure cannot be decided from these data. The writers hope to repeat this experiment under rigidly controlled temperature at or near the optimum for germination.

\* Samples of freshly collected conidia of the *Erysiphaceae* usually contain a few germinating spores. In the present work a sample was discarded if, upon examination, it was found to have more than 0.5 to 1% of spores that had germinated before being subjected to experiment.



### C—COMPARISON OF TIME REQUIRED FOR GERMINATION OF CONIDIA UNDER CONDITIONS OF REDUCED AND ATMOSPHERIC PRESSURE RESPECTIVELY

When it had become evident that a slight reduction in air pressure brought about an enhancement of germination of mildew conidia, the question arose: does the pressure reduction actually increase the number of spores that will germinate on a given slide, i.e. does it induce spores to germinate that might otherwise fail to do so, or is the effect of pressure reduction merely to hasten the germination process so that more spores germinate within the three hours time limit of the experiment?

The data of Table V show that, at room temperature, conidia germinate more rapidly under reduced pressure (125 mm. below atmospheric pressure) than they do at atmospheric pressure and from Table VI it may be seen that essentially the same result was obtained at lower temperature.

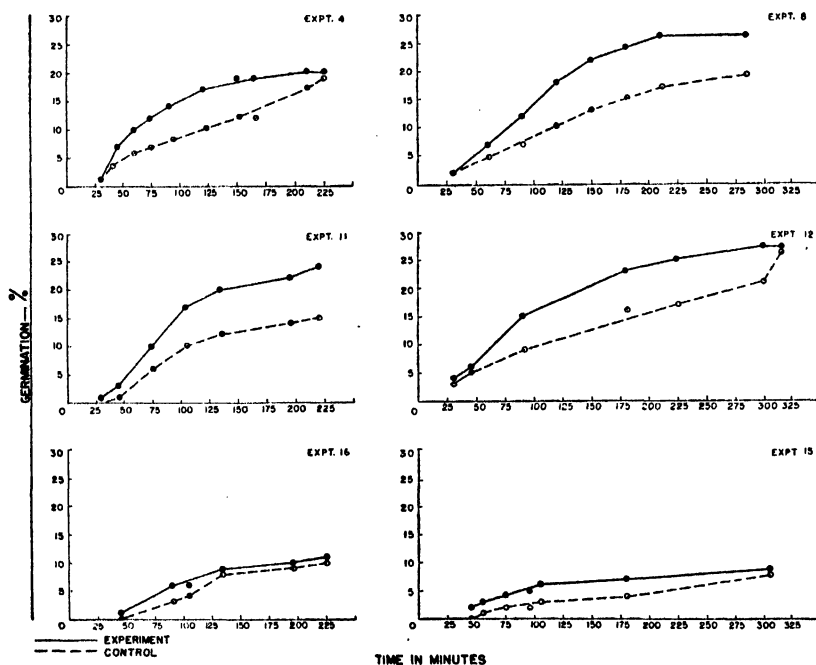


FIG. 6. Graphs showing differences in rate of germination of conidia of *Erysiphe graminis* Hordei at atmospheric pressure (broken lines) and reduced pressure (solid lines): Expts. Nos. 4, 8, 11, 12 at room temperature; Expts. Nos. 15 and 16 at 8° C.

Referring to Table V, it will be seen that, in all experiments, germination at reduced pressure was considerably higher than at atmospheric pressure *at the end of three hours*, which is in accord with the results presented in Part A of the section dealing with data. That is, when conidia are subjected to reduced and to atmospheric pressure, respectively, one obtains a higher germination percentage at reduced pressure because more spores have germinated under

the latter conditions during the three hour period of the experiment. However, when germination is allowed to proceed for a much longer time, e.g. for three hours, 40 min. in Expt. 4 (Table V) and for five hours, 15 min. in Expt. 12 (Table V), the germination at atmospheric pressure tends to become equal to that at reduced pressure. This is seen graphically in the tendency of the curves (Fig. 6, Expts. 4, 12, 15) to meet.

One curious fact may be noted. Not in all experiments did the spores at atmospheric pressure 'catch up' to those at reduced pressure. It was usually observed that, for some unknown reason, after four hours all the spores that were able to germinate in a sample had germinated. There were, however, always some that failed to germinate despite their turgid and otherwise normal appearance.

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NUMBER 1

## GROWTH AND SURVIVAL OF BACTERIA IN PEAT

### I. POWDERED PEAT AND RELATED PRODUCTS<sup>1</sup>

BY A. G. LOCHHEAD<sup>2</sup> AND R. H. THEXTON<sup>3</sup>

#### Abstract

Comparative tests of various powdered materials showed that well humified peat was superior to other preparations in maintaining the test bacteria in viable condition. Under the test conditions, sterilization of peat improved it as a medium for the maintenance of high numbers of viable cells. Neutralization was important, and more satisfactory following, than preceding, sterilization. Potassium carbonate was more suitable than sodium carbonate as neutralizing agent for promoting longevity of bacteria. Though peat allowed to dry immediately after inoculation did not permit the same high increase in bacterial numbers observed in peat kept at suitable moisture content, it was able to maintain large numbers of organisms in viable condition. Peat allowed to dry following the initial rise continued to support an increased load of living cells. After the initial increase at growth temperatures, peat stored at 4° C. maintained numbers of bacteria better than at the higher temperature. Though immediate storage at 4° C. resulted in a pronounced decline in numbers, subsequent removal to a temperature suitable for growth, even though delayed for as long as 12 wk., was followed by a rapid increase of bacteria to very high numbers.

#### Introduction

The question of employing suitable carriers for bacteria or other microorganisms is often an important matter in industrial and agricultural microbiology. Though in the majority of industrial fermentation processes an inoculum for larger scale cultivation can be suitably prepared in liquid media prior to use, in other cases it is of advantage or even imperative to have the organism maintained in such condition that it may be used after a period of storage to provide an effective inoculum that can be used without further manipulation. This is of importance, for example, in such diverse applications as seed inoculation with various species of *Rhizobium*, the seeding of cheese curd with *Penicillium roqueforti*, or the application to soil of bacteria pathogenic for larvae of destructive insects, as illustrated by the use of *Bacillus popilliae* for the control of the Japanese beetle (2).

From the standpoints of convenience in handling and effective dispersal of the inoculum throughout the material to be treated, a dry carrier has many advantages over a liquid inoculum. Liquid cultures, moreover, have notor-

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iously poor longevity, the viability and effectiveness of the organism being adversely affected by accumulated metabolic products. They are, therefore, not well adapted as carriers requiring storage. The preparation of cultures in lyophile form, though excellent for the purpose of preservation, is less suited to use in larger scale inoculation procedures than cultures maintained in a carrier adapted to more effective dispersion for immediate use.

Various materials have been used as dry carriers for bacteria. Considerable success has been attained in the use of dried skim milk powder as carrier for lactic and propionic acid bacteria for use in dairy manufacturing processes, following the work of Rogers (6). Talc and calcium carbonate have been used by Dutky (1, 2) as carriers for spores of *B. popilliae* in testing the value of this organism in controlling soil-inhabiting insect larvae. In recent years considerable interest has been shown in developing legume seed inoculants consisting of different species and strains of *Rhizobium* carried on sand, soil, charcoal, or humus-rich material such as peat, with the carrier prepared in varying degrees of fineness and with varying moisture content. Though not all have proved efficient, certain inoculants, particularly those of the humus type, have supplanted to a large extent the older, but effective, agar cultures, in view of the greater ease of application.

In studying the longevity of non-spore-forming bacteria in air-dry soil, Giltner and Langworthy (3) concluded that survival is due, not only to retention in soil of moisture in hygroscopic form, but also to the presence, in the richer soils, of soluble organic constituents that have a protective influence on bacteria subjected to desiccation. From such considerations even more favourable conditions for survival might be expected to obtain in peat and related substances rich in organic matter in various stages of decomposition. Moreover, the high adsorptive capacity of such materials suggests a further value for use as carriers through their capacity to immobilize, at least partially, by-products of bacteria metabolism. An additional advantage lies in their relatively light weight, making them, in finely powdered form, adapted to more thorough mixing or covering of the material to be inoculated.

The present report includes comparative tests on the suitability of different grades of peat and lignite as carriers for bacteria, with special attention to the former. Supplies were furnished through courtesy of the Division of Fuels, Department of Mines and Resources. The peat was obtained from sub-surface layers of the Alfred, Ont., deposit and represented humified sphagnum that was air-dried and ground for use. It was dark brown in colour. The lignites, darker in colour than the peat, were obtained from Northern Ontario deposits.

### Experimental

To compare the suitability of various carriers, preliminary tests were made on the following materials:

Charcoal—fine powder (No. 24)

Earthy lignite—fine powder ('54% minus 200 mesh')

Woody lignite—fine powder ('57% minus 200 mesh')

Peat—fine powder ('48% minus 200 mesh')

Peat—medium fine ('minus 1/32 in. mesh')

Peat—medium coarse ('minus 1/16 in. mesh')

Peat—coarse ('on 1/16 in. mesh')

The moisture content, pH value, and bacteria counts of the air-dry materials as received are shown in Table I. The finer powders could take up 30% or more of moisture and still remain in 'dry' condition. The object of the

TABLE I

MOISTURE, pH, AND BACTERIA COUNTS OF AIR-DRY CARRIERS

	Moisture, %	pH	Bacteria count per gm.	
			Nutrient agar, 28° C., 3 days	Soil extract agar 28° C., 10 days
Charcoal	4.0	8.5	175,000	350,000
Earthy lignite	9.6	6.4	27,000	157,000
Woody lignite	9.6	5.0	190,000	172,000
Peat—fine	11.1	4.6	55,000	165,000
Peat—medium fine	11.1	4.5	130,000	225,000
Peat—medium coarse	11.0	4.4	48,000	49,000
Peat—coarse	9.7	4.8	32,000	41,000

experiments was to determine the numbers of viable organisms on inoculated carriers after periods of storage, and the tests included studies of the effect of sterilization, neutralization, holding temperature, desiccation, and various addenda to the materials. The high adsorptive capacity of peat and related materials for by-products of microbial growth suggested their value, not only as carriers proper (i.e. by mixing with high numbers of organisms, which it is desired to maintain), but as a medium for development of the inoculated bacteria, thereby simplifying the manner of preparation.

As test organisms, non-sporing bacteria, not indigenous to soil or peat, and therefore presumably not specially favoured over autochthonous soil types, were selected. *Salmonella typhimurium* and *Escherichia coli* were used, these organisms having the advantage of being adapted to ready quantitative estimation by plating methods on selective media. It was also felt that results from tests with these species would serve as a starting point for studies on a wider variety of organisms that might be found suited to use in the control of fungal or insect pests.

For analysis 10-gm. samples of material (usually in duplicate) were weighed out aseptically and transferred to a 100 ml. Erlenmeyer flask containing 90 ml. of sterile tap water and fitted with a rubber stopper. The flask was then shaken for five minutes and further serial dilutions were prepared as required and shaken one minute. Appropriate dilutions were transferred to Petri

plates (in triplicate) and plates poured with violet red bile agar (Difco). For *E. coli*, plates were counted after incubation for 24 hr. at 37° C., and in the case of *S. typhimurium*, after 48 hr. This medium was found very suitable for both organisms, giving characteristic dark colonies. At frequent intervals colonies were picked from the plates and compared with the original culture to assure identity.

### Comparison of Various Carriers

In the preliminary test 10 carriers were compared—charcoal, earthy lignite (neutralized), and four grades of peat, each of which was tested without being neutralized and after neutralization with sodium carbonate. The materials were first sterilized by autoclaving at 15 lb. pressure for four hours. Neutralization was done by addition of sufficient sodium carbonate to adjust the pH to 6.8 to 7.0. In the case of fine peat with pH of 4.5, 15 ml. of 20% sodium carbonate solution per 100 gm. of peat was usually satisfactory. (Later tests indicated that potassium carbonate was superior to sodium carbonate.) Extra water required to bring the moisture content to 30% was added, allowance being made for the moisture added in the inoculum. For this and most tests a 48 hr. culture of the organism in nutrient broth was added, usually 15 to 20 ml. per 100 gm. of dry powder, and well mixed with the carrier, which was then ready for storage.

Comparative tests of carriers adjusted to 30% moisture, inoculated with *E. coli*, packed in screw-capped containers and stored at room temperature, are summarized in Table II. The results show the superiority of the neutralized peat over the other carriers, and particularly the effect of neutralization on the suitability of peat itself (Fig. 1). Charcoal and lignite, though permitting increases in bacteria up to two weeks, were less satisfactory than the neutralized peats, the numbers failing to reach such high levels and showing an earlier decline.

TABLE II

COMPARISON OF VARIOUS CARRIERS FOR BACTERIA

(INOCULATED WITH *Escherichia coli*, ROOM TEMPERATURE STORAGE, MOISTURE 30%, BACTERIA COUNTS IN MILLIONS PER GRAM)

Length of storage	Char-coal	Earthy lignite	Fine peat		Medium fine peat		Medium coarse peat		Coarse peat	
			Not neutr.	Neutr.	Not neutr.	Neutr.	Not neutr.	Neutr.	Not neutr.	Neutr.
At start	36	36	36	36	36	36	36	36	36	36
1 day	270	125	8.7	330	14	140	19	320	14	650
2 weeks	500	325	1.2	1160	5.4	1290	16	650	22	360
1 month	320	280	1.2	920	7.9	790	18	230	23	780
2 months	115	117	1.5	570	6.3	690	4	140	13	230
4 months	17	10	0.09	210	1.3	205	10	90	9.8	130
6 months	4	3.4	0.008	3.7	0.05	6.1	0.3	3.1	0.2	3.5
9 months	0.7	0.3	0.0003	0.3	0.01	0.7	0.1	0.03	0.03	0.03

A further comparative test was made with the finely powdered materials used as carriers for *S. typhimurium*. Charcoal, earthy lignite, woody lignite, and peat, all neutralized and with a moisture content adjusted to 25%, with

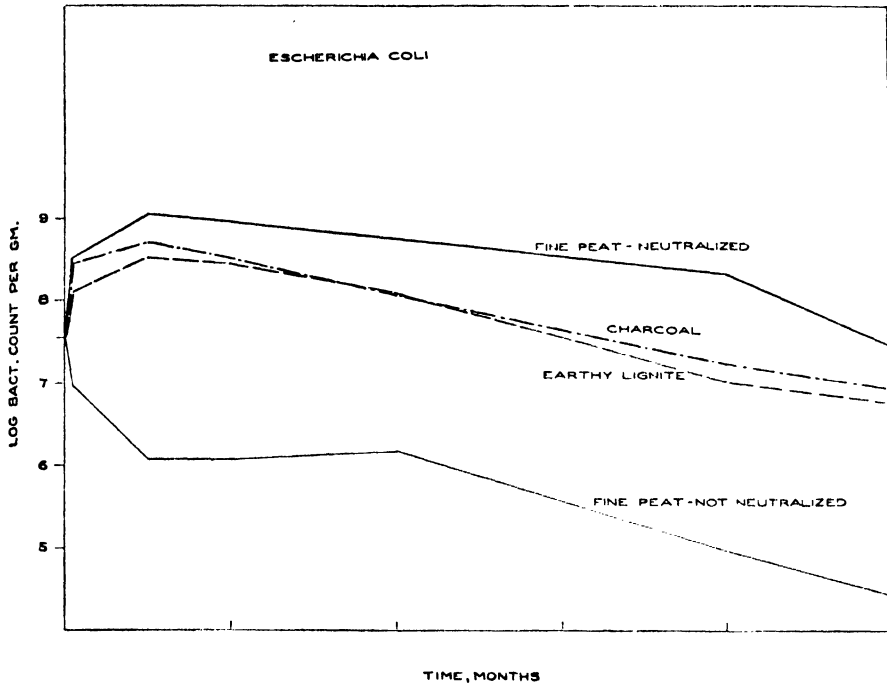


FIG. 1. Survival of *Escherichia coli* on various carriers in powder form.

an additional lot of peat with 30% moisture, were held at room temperature for six months in closed containers. The results (Fig. 2) again showed peat to be superior to the other carriers. With an original count of 26 and 24.5 million cells per gram respectively, the two peat carriers gave maximum numbers of organisms between two weeks' and one month's storage, with viable counts of 36.2 and 21.4 millions per gram after six months' holding. In view of the superiority of peat over the lignites and charcoal, subsequent experiments were confined to peat.

### Effect of Moisture Content

Further information on the effect of the moisture content on the development and survival of bacteria added to powdered peat was obtained in a test with *E. coli*. Different portions of the carrier were prepared to contain 20, 25, and 30% moisture respectively and after inoculation were stored at room temperature in closed containers. As indicated in Fig. 3, little difference was noted between lots packed at 25 and 30% moisture (cf. also Fig. 2). At 20% moisture, however, conditions for preliminary increase were less satisfactory and numbers of viable organisms remained at lower levels.



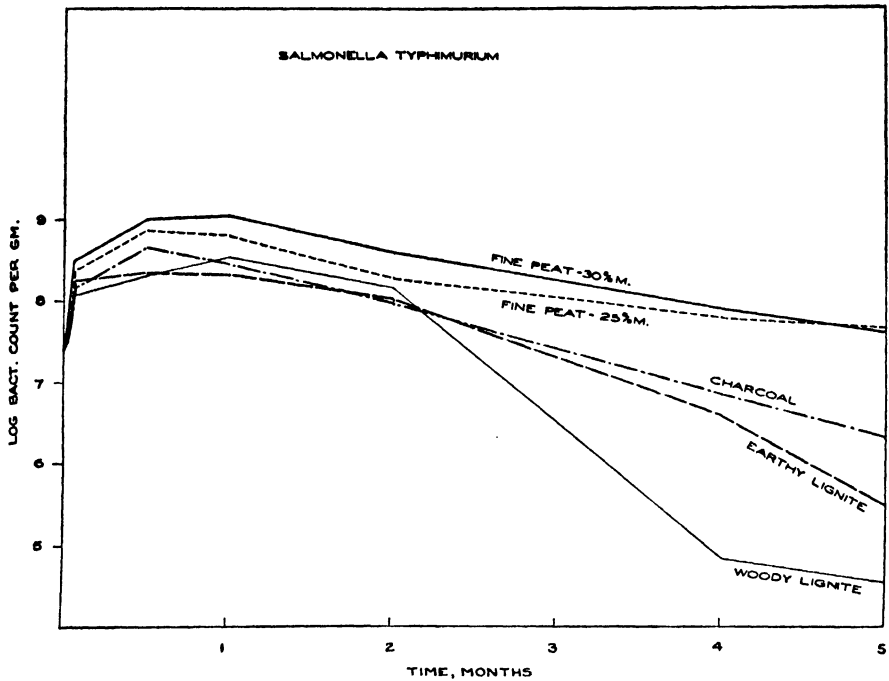


FIG. 2. Survival of *Salmonella typhimurium* on various carriers in powder form.

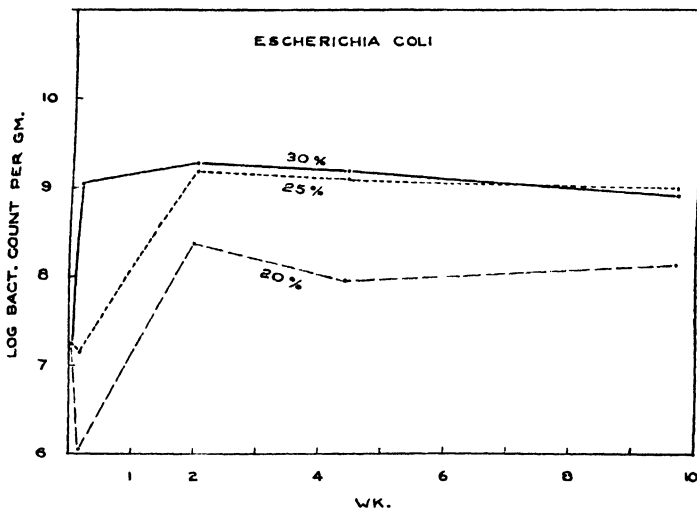


FIG. 3. Effect of moisture content on numbers of *Escherichia coli* in powdered peat.

### Effect of Age of Inoculum

To note the effect of the age of the culture used for inoculation, a number of comparative tests were made with fine peat inoculated with 24-hr. and 48-hr. cultures respectively of *S. typhimurium*. Results are illustrated by data

from two tests summarized in Table III. The findings indicate that under the experimental conditions the age of the inoculum was unimportant with the test organism used.

TABLE III

EFFECT OF AGE OF INOCULUM ON SURVIVAL OF *Salmonella typhimurium* IN FINE PEAT  
(BACTERIA COUNTS IN MILLIONS PER GRAM)

Storage	Stored at 15°-20° C.		Stored at 26° C.	
	Age of inoculum, hr.			
	24	48	24	48
At start	36	19	17	19
After 1 week	690	600	900	1140
After 2 weeks	610	510	1540	1500
After 3 weeks			1670	1090
After 4 weeks	485	490	1480	1460

### Effect of Addition of Milk, Gelatine, or Agar

In the procedure adopted, the only addition of bacterial nutrient material to the carrier was that contained in the inoculating fluid. To test the effect of incorporating other substances, an experiment was made in which skim milk (0.5%), gelatine (0.05%), and agar (0.025%) were added respectively in dilute solutions to the carriers. The results, shown in Fig. 4, indicated that under the conditions of the test the incorporation of these materials was without significant effect.

### Effect of Sterilization and Neutralization

Various observations have indicated that humus contains substances that may be injurious or favourable to the growth of higher plants and also of certain micro-organisms (7). Though little of an exact nature is known of such substances, it is believed that they may be liberated, not only as transformation products of the decomposition of plant residues, but also as products synthesized by micro-organisms or formed following their death and eventual decomposition. Lochhead and Chase (5) have shown that soil contains heat-stable growth factors required by certain groups of soil bacteria, some of which growth-promoting substances may be synthesized by other soil bacteria having simpler nutritional requirements. On the other hand soil may contain substances toxic to bacteria, some of which may be destroyed by heat. Among such antibacterial substances are those earlier described as bacterio-toxins (4) and those recognized as related to antibiotics, following the demonstration by Waksman and Woodruff (8) of the occurrence in soil of substances of the actinomycin type. Since growth-promoting and growth-inhibiting substances may both be present, experiments were made to note the effect of sterilization

on the effectiveness of fine peat as a carrier. A summary is presented in Table IV, which includes results from comparative tests with potassium carbonate and sodium carbonate as neutralizing agents.

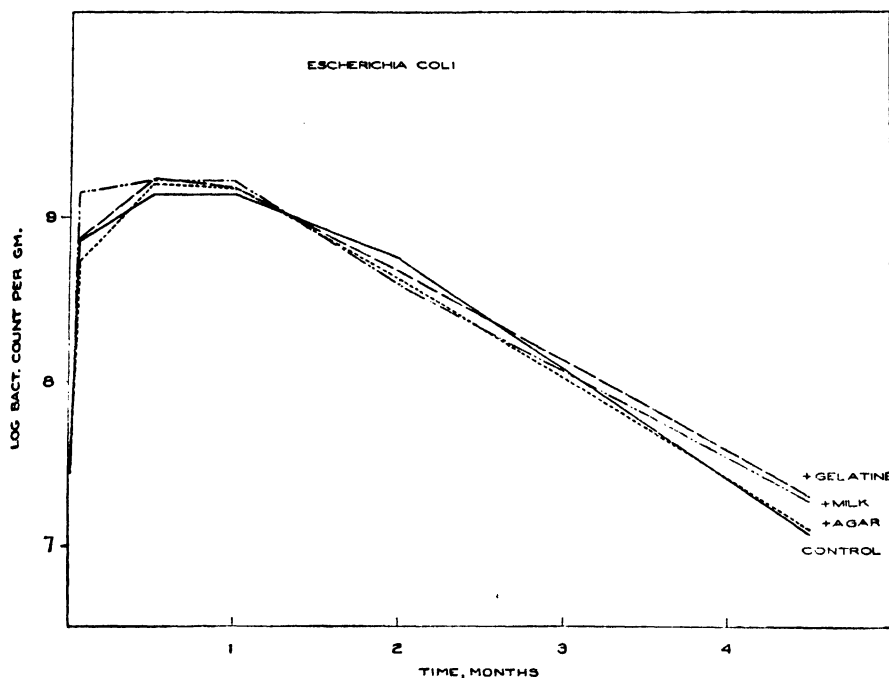


FIG. 4. Effect of adding gelatine, milk, or agar to powdered peat as carrier for *Escherichia coli*.

From these, as well as other tests, the data indicate that sterilization (autoclaving) of the powdered peat used improves it as a medium for supporting growth of certain bacteria introduced into it. To what extent this effect is to be ascribed to a liberation of substances favouring growth, to the removal of toxic substances, or to the destruction of antagonistic micro-organisms is uncertain. Table IV shows also that neutralization should follow, rather than precede, sterilization, and that potassium carbonate is superior to sodium carbonate as neutralizing agent. This was noted also in smaller scale tests in which peat was inoculated with *E. coli*.

### Effect of Desiccation

Certain conditions under which bacterial carriers might be used, such as the direct application to soil for pest control, would make them subject to desiccation through exposure to air in thin layers. To test the viability of *S. typhimurium* under such conditions, an experiment was made in which inoculated fine peat, exposed to air in a shallow layer of approximately  $\frac{1}{2}$ -in. depth, was compared with similar material kept in closed tins containing approximately 400 gm. of peat. A third lot was held for one week in a closed

TABLE IV

EFFECT OF NEUTRALIZATION AND STERILIZATION ON SURVIVAL OF *Salmonella typhimurium* IN FINE PEAT

(BACTERIA COUNTS IN MILLIONS PER GRAM)

Storage period	Temp. of storage	Sterilized, neutralized with $K_2CO_3$	Sterilized, neutralized with $Na_2CO_3$	Not sterilized, neutralized with $Na_2CO_3$	Neutralized with $Na_2CO_3$ before sterilization
At start	15°–20° C.	35	41	41	41
1 day		80	84	28	49
1 week		180	390	146	178
2 weeks		1060	460	160	27
4 weeks		1130	245	82	10
6 weeks		990	225	70	—
At start	26° C.	19	19	19	
1 day		202	69	71	
1 week		1300	1140	1070	
2 weeks		2290	1500	950	
3 weeks		1570	1090	650	
4 weeks		1840	1460	590	
At start	20° C. $\pm$	33	47		
2 days		1280	700		
1 week		1670	630		
2 weeks		3130	730		
3 weeks		2450	710		
4 weeks		1620	470		
5 weeks		2330	480		
7 weeks		1870	155		
10 weeks		1470	100		

container to allow for a preliminary increase in bacterial numbers before being exposed to air in a shallow layer. Data for bacterial counts and moisture throughout a 10 wk. storage period at room temperature are shown in Fig. 5.

Though immediate exposure to drying prevented an initial rise in numbers comparable to that in the unexposed peat, considerable numbers of viable organisms remained at a comparatively even level. However, when peat was kept for one week after inoculation in the closed containers before being exposed to desiccation, much higher numbers of bacteria were not only reached, but also maintained throughout the 10 wk. period. Though the moisture content decreased rapidly, and became identical with that of the peat exposed immediately after inoculation, no corresponding reduction in counts to similar levels was noted (530 million as compared with 68 million, viable count per gram, after 10 wk.). The results, though indicating that dried powdered peat at low moisture content was less suited to the increase of bacteria, demonstrated a rather remarkable ability of the dried carrier to maintain in viable condition large numbers of bacteria, and to continue to carry an increased load of bacteria in living condition.

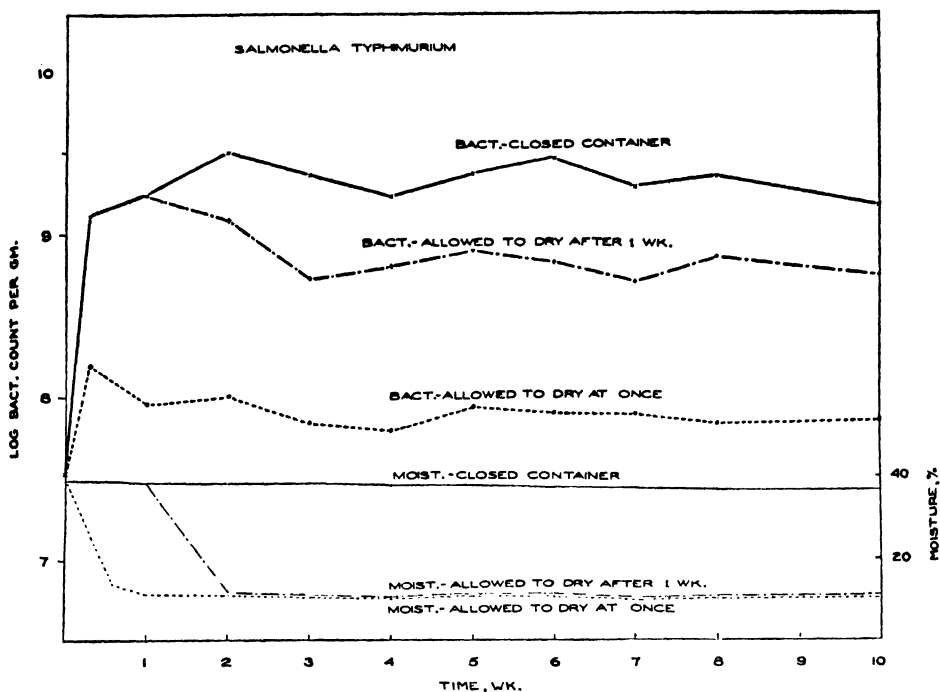


FIG. 5. Effect of desiccation on survival of *Salmonella typhimurium* in powdered peat.

### Studies on Storage Temperature

When powdered peat of suitable moisture content is inoculated with *S. typhimurium* or *E. coli* and held at moderate temperatures, there is normally a rapid increase in numbers which reach their peak usually within one or two weeks, after which a gradual decline sets in. A series of tests was undertaken to note the effect of altering storage temperatures so as to provide for higher numbers of bacteria in older material. The tests included comparative studies of:

- Continuous storage at room temperature (approx. 20° C.).
- Continuous storage at low temperature (4° C.).
- Preliminary storage at room temperature for two weeks (to allow for initial increase), followed by storage at 4° C.
- Preliminary storage at low temperature (before normal increase has taken place) for various periods, followed by storage at room temperature.

Data for experiments with *S. typhimurium* covering a period of 14 wk. and with *E. coli* during 10 wk. storage are presented graphically in Figs. 6 and 7. It is noted that whereas at room temperature there is a characteristic rapid increase, storage at a temperature of 4° C. shows no such rise, so that numbers of organisms are at all times much lower. It is of interest to observe, however,

that after two weeks at room temperature, removal to storage at the lower temperature results in a slower decline in numbers than continuous holding at room temperature.

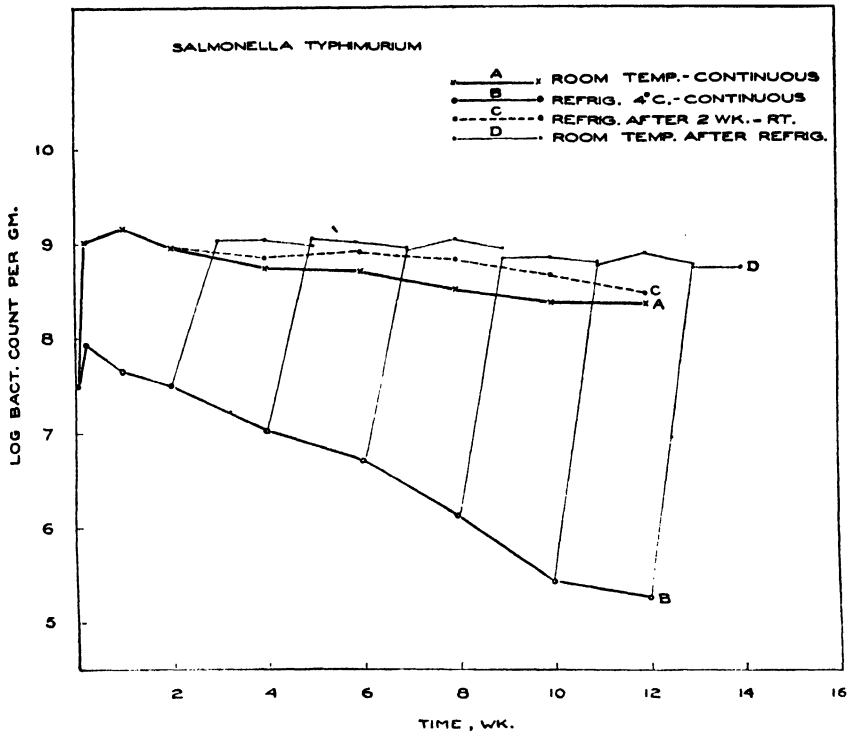


FIG. 6. Temperature storage conditions in relation to survival of *Salmonella typhimurium* in powdered peat.

Results from tests on samples placed at room temperature after varying periods at 4° C. are of special interest in showing that the capacity for rapid increase at suitable growth temperature is well maintained during storage at low temperature for the duration of the experiments. As a result, refrigerated samples brought to room temperature may contain, as in the case of *S. typhimurium*, considerably higher numbers of viable bacteria at any given time than other samples of the same age. This ability to increase rapidly to high numbers (in the region of  $10^9$  per gram) was shown by samples in which refrigeration had reduced the original count to a comparatively low figure (e.g. 180,000 cells of *S. typhimurium* per gm. after 12 wk., or 185,000 cells of *E. coli* per gm. after eight weeks). This confirms general observations made throughout the studies suggesting that within fairly wide limits the number of organisms in the inoculum is not an important factor in determining the extent to which bacteria will increase. When numbers have fallen below a minimum for good recovery, as indicated by a count of 2000 cells of *E. coli* per gm. after 10 wk. at 4° C., subsequent increase at 20° C. is less

pronounced, though numbers rise to 170 million per gram after one week's transfer to the more favourable temperature (cf. Fig. 7).

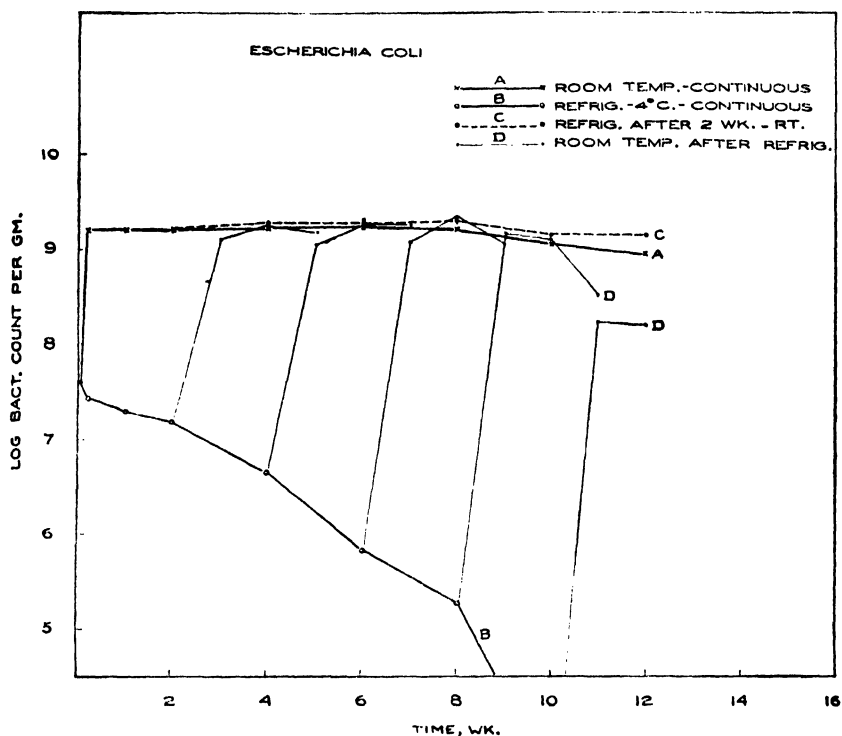


FIG. 7. Temperature storage conditions in relation to survival of *Escherichia coli* in powdered peat.

### Growth Promoting Properties of Peat

Since, as has been referred to above, humus-rich material such as the well-decomposed peat employed may contain substances that may exert either favourable or unfavourable effects on the growth of plants or micro-organisms, it is probable that the effectiveness of peat as a medium for promoting growth and longevity of bacteria will vary with the specific organisms under test. In view of the apparent value of the type of powdered peat used in permitting relatively large numbers of the test bacteria to remain viable during storage, some tests were made to note the effect of peat extracts on bacterial development.

For the tests, a synthetic liquid medium was prepared containing mineral salts and sodium citrate as used in Simmons' citrate agar. In this basal medium were incorporated increasing quantities of peat extract, different series of tubes being inoculated uniformly with cultures of various organisms. Two extracts of peat were used, prepared respectively by autoclaving peat with water and by extracting with water without heating. Before use the extracts were brought to neutrality with sodium hydroxide.

TABLE V  
EFFECT OF EXTRACTS OF PEAT ON GROWTH OF VARIOUS BACTERIA

Treatment of peat	Test organism	Medium	Time (hr.)	Peat extract per 10 ml. medium				
				0	0.05	0.1	0.5	1.0
100 gm. peat + 300 ml. water autoclav., filt., and neutralized	<i>S. typhimurium</i>	Minerals-citrate	24	++	++	+++	++++	
	<i>S. schottmülleri</i>	Minerals-citrate	24	++	+++	+++	++++	
Cold water extract (100 gm. peat + 200 ml. water) neutralized	<i>S. typhimurium</i>	Minerals-citrate	24	++	++	++	++	++
	<i>S. schottmülleri</i>	Minerals-citrate	24	+	+	+	++	++
	<i>Aerobacter aerogenes</i>	Minerals-citrate	24	++	++	++	++	++
	<i>Micrococcus</i> sp. (soil)	Minerals-citrate	24	++	+++	+++	++++	++++
	<i>Corynebacterium</i> sp. (soil)	Minerals-citrate	24	++	+++	+++	++++	++++
	<i>Bacterium</i> sp., (Mn-oxidizing soil)	Minerals-citrate	72	+	+	+++	+++	+++

From Table V it is noted that the addition of peat in the amounts used exerted a generally stimulating effect on the growth of the test organisms, with no depressing action observed. Although the tests were limited in scope they suggest that for some species of bacteria well humified peat contains growth promoting substances. Further investigation is required to elucidate their nature.

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## GROWTH AND SURVIVAL OF BACTERIA IN PEAT

### II. PEAT PELLETS<sup>1</sup>

By A. G. LOCHHEAD<sup>2</sup> AND R. H. THEXTON<sup>3</sup>

#### Abstract

Pellets prepared from spongy, unhumified peat, were found to be well suited to the growth of the test organisms and were superior to pellets made from compressed, well humified peat. Sterilization had the effect of lowering somewhat the absorptive capacity of the spongy pellets and increased the buffering capacity. Neutralization was essential for sterilized pellets in maintaining viable organisms, though it was much less important with unsterilized peat. In contrast to powdered, humified peat, pellets of spongy peat showed to best advantage when allowed to become air-dry immediately following inoculation. In pellets subjected to immediate desiccation, the test organisms attained a much greater development, and were maintained in greater numbers than in peat kept moist or when desiccation was delayed.

#### Introduction

In a previous report (1) it was shown that powdered peat, prepared from the more compact, well humified layers of a sphagnum bog deposit, was superior to certain other powdered materials as a carrier for maintaining the test bacteria in viable condition for considerable periods of time. In view of the possibility that a material in the form of small pellets might possess certain advantages over fine powder by affording better protection to bacteria exposed to desiccation, additional studies were made with different types of peat in pellet form.

#### Experimental

Tests were conducted with pellets prepared from two types of peat, as follows:

(a) Hard, compressed peat, prepared from well humified material from which the powdered peat previously studied was obtained.

(b) Spongy peat from unhumified surface deposits, light brown in colour, slightly compressed but very light and porous.

For the experiments, the hard peat was sawn, and the spongy peat cut, into cubes of approximately  $\frac{1}{8}$  to  $\frac{3}{8}$  in. (Fig. 1). The cubes of hard peat weighed approximately 0.3 gm. each, and those of spongy peat, 0.05 to 0.06 gm., and gave pH values, when ground up, of 4.7 and 3.8 to 4.3, respectively, the latter being less homogeneous in texture.

Neutralization was done by soaking the pellets in potassium carbonate solution followed by a rinsing in water, after which they were inoculated by immersion for one hour in a 24 hr. culture of the bacteria. They were then

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allowed to drain and then placed in storage. The test organism was, in most cases, *Salmonella typhimurium*.

For analysis, 5 to 10 pellets were shaken in sterile tap water and appropriate dilutions plated out, in triplicate, with violet red bile agar. This procedure was felt to be more suitable, from the standpoint of practical significance, than grinding the pellet, giving a measure of the bacteria removable by shaking. After 48 hr. incubation at 37° C. plates were counted and numbers of bacteria estimated per pellet or per gram.

### Comparison of Hard and Spongy Pellets

Preliminary experiments were made with the two types of peat, the tests being restricted to a study of the effect of immersing the pellets, prior to inoculation, in various solutions to note any influence upon the numbers of viable organisms removable by shaking. Before the treatments the hard pellets were 'neutralized' to pH 6.8 by soaking them in 5% potassium carbonate solution for 40 min., and the spongy pellets immersed in 1% potassium carbonate for 20 min. The pellets were allowed to remain for 16 hr. in the respective test solutions, after which the solutions were drained off and replaced by the inoculum.

The results of several experiments, illustrated by data summarized in Table I, did not provide any significant findings as far as the treatments were concerned. Further tests with such treatment were therefore dispensed with. However, the spongy peat pellets showed to considerable advantage over the hard peat; doubtless this was attributable to the much greater porosity of the former, increased surface, and greater absorptive capacity. In the case of the hard peat, the numbers of viable organisms removable by shaking had

TABLE I

EFFECT OF VARIOUS TREATMENTS OF PEAT PELLETS ON NUMBERS OF BACTERIA REMOVED BY SHAKING

(*S. typhimurium*, STORAGE AT 26° TO 30° C.)

Treatment	Hard pellets. Bacteria removed by shaking, per pellet (millions)				Spongy pellets. Bacteria removed by shaking, per pellet (millions)			
	Days				Days			
	2	7	14	21	2	7	14	21
Glycerine	1040	530	80	13	4560	830	195	84
Dextrin	503	190	55	38	1980	1080	286	435
Glycerine + dextrin	1410	950	183	43	4270	1020	295	49
Dextrin + K <sub>2</sub> HPO <sub>4</sub>	370	196	12	7	—	—	—	—
Sodium glycerophosphate	—	—	—	—	2180	980	380	273
Gum tragacanth	—	—	—	—	1180	705	250	198
Cysteine + growth factors	337	238	15	5	—	—	—	—
Water	530	145	45	19	870	1100	470	290

declined to figures lower than those considered desirable for a bacterial carrier. Consequently further tests were confined to the spongy peat.

### Effect of Sterilization and Neutralization

The exact control of pH in spongy peat presented some difficulty due to the fact that the material was not homogeneous, but still fibrous in nature. It was also noted that sterilization resulted in a somewhat lowered capacity for absorbing liquid. Unsterilized pellets were able to absorb approximately 15 times, and sterilized pellets about 10 times, their weight of water. On drying, moisture was rapidly lost, the pellets becoming air-dry after three or four days and retaining, in this state, about 8% moisture.

Sterilization was found to increase considerably the buffering capacity of the spongy peat. For unsterilized pellets, immersion for 20 min. in 0.5% potassium carbonate solution was found to be sufficient for neutralization, while after sterilization an exposure of 60 min. in 1% solution was required. It was also noted that neutralization of unsterilized peat of this type was not required for the development of *S. typhimurium*, the simple addition of the broth cultures used as inoculum being sufficient to bring the pH to a point

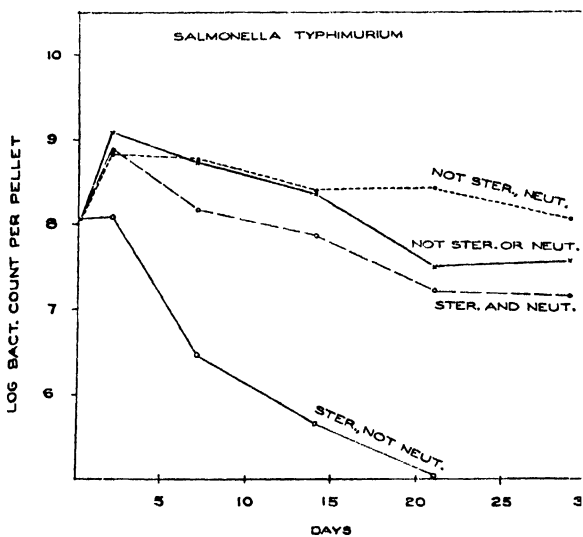


FIG. 2. Neutralization and sterilization of spongy peat pellets in relation to survival of *Salmonella typhimurium*.

suitable for the organisms (pH = 7.0 approximately, after two days). On the other hand, sterilized peat was not similarly affected (pH = 5.4 approximately, after two days), and required neutralization to provide conditions suitable for growth. The relation of sterilization to neutralization in affecting the survival of organisms carried on spongy peat is illustrated in Fig. 2.

Further data on the effect of sterilization and neutralization are shown in Fig. 3. Comparative tests were made with *S. typhimurium* and *Shigella*

*alkalescens* under conditions that permitted more rapid desiccation of the inoculated material than in the previous experiments. The numbers of viable bacteria are expressed on the 'per gram' basis, permitting a better comparison of the spongy unhumified peat with powdered humified peat as carrier, previously reported (1). Higher numbers of viable organisms (in excess of  $10^{10}$  per gram) were found in the spongy peat than in powdered peat

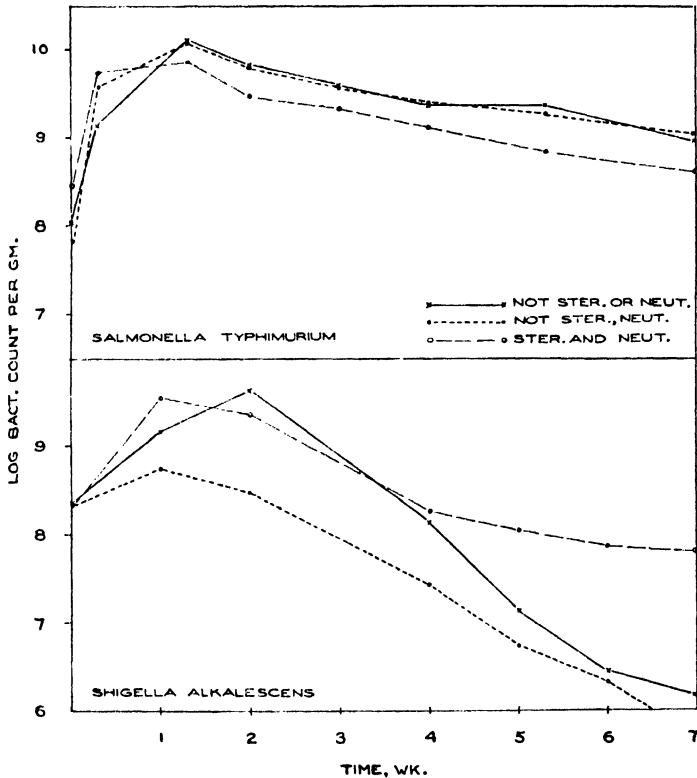


FIG. 3. Survival of *Salmonella typhimurium* and *Shigella alkalescens* on spongy peat pellets.

within storage periods of three weeks. After storage for seven weeks, the numbers of *S. typhimurium* compared favourably with those found in the powdered material, the unsterilized samples, whether neutralized or not, being as suitable as the sterilized and neutralized peat. *Shigella alkalescens* showed poorer viability than *S. typhimurium*, though in the sterilized and neutralized pellets counts in the neighbourhood of  $10^8$  per gram were recorded after six to seven weeks.

The importance of sterilization doubtless varies, not only with the organism being carried, but also with the nature of the contaminants in the raw material. Though the use of an unsterilized carrier may be, at first thought, repellent, it should be kept in mind that in larger scale production strict bacteriological sterility is difficult to achieve and maintain. In addition, there is some

TABLE II

EFFECT OF DESICCATION ON NUMBERS OF VIABLE BACTERIA IN PELLETS OF SPONGY PEAT  
(*S. typhimurium*, STORAGE AT 26° C.)

Treatment and storage	Allowed to dry immediately after inoculation			Closed containers			Closed containers one week, then allowed to dry		
	pH	Average weight, gm.	Bacteria per gram (millions)	pH	Average weight, gm.	Bacteria per gram (millions)	pH	Average weight, gm.	Bacteria per gram (millions)
<b>Not sterilized or neutralized</b>									
Fresh pellets	3.8	0.056	—	—	—	—	—	—	—
After inoculation	5.0	0.747	132	5.0	0.747	132	—	—	—
Stored 2 days	6.5	0.456	2280	6.9	0.713	1420	—	—	—
Stored 1 wk.	7.1	0.057	12,280	7.6	0.719	2380	—	—	—
Stored 2 wk.	6.8	0.056	3800	8.0	0.656	1030	6.9	0.053	1530
Stored 3 wk.	6.4	0.055	2510	7.8	0.638	72	6.4	0.052	680
Stored 4 wk.	6.3	0.057	760	7.8	0.653	0.63	6.5	0.052	114
Stored 5 wk.	6.2	0.057	300	7.6	0.530	0.15	6.1	0.050	8.6
<b>Sterilized and neutralized</b>									
Fresh pellets	3.8	0.056	—	—	—	—	—	—	—
After ster. and neutr.	6.9	0.346	—	—	—	—	—	—	—
After inoculation	6.7	0.522	470	6.7	0.522	470	—	—	—
Stored 2 days	7.4	0.265	2450	7.2	0.533	1760	—	—	—
Stored 1 wk.	7.1	0.051	6690	7.5	0.547	2670	—	—	—
Stored 2 wk.	7.1	0.050	2960	8.2	0.541	1550	7.0	0.050	1420
Stored 3 wk.	6.4	0.051	2670	8.1	0.529	64	6.7	0.049	670
Stored 4 wk.	6.3	0.049	810	7.3	0.564	0.69	6.6	0.045	155
Stored 5 wk.	6.2	0.049	990	7.8	0.511	0.04	6.5	0.047	38

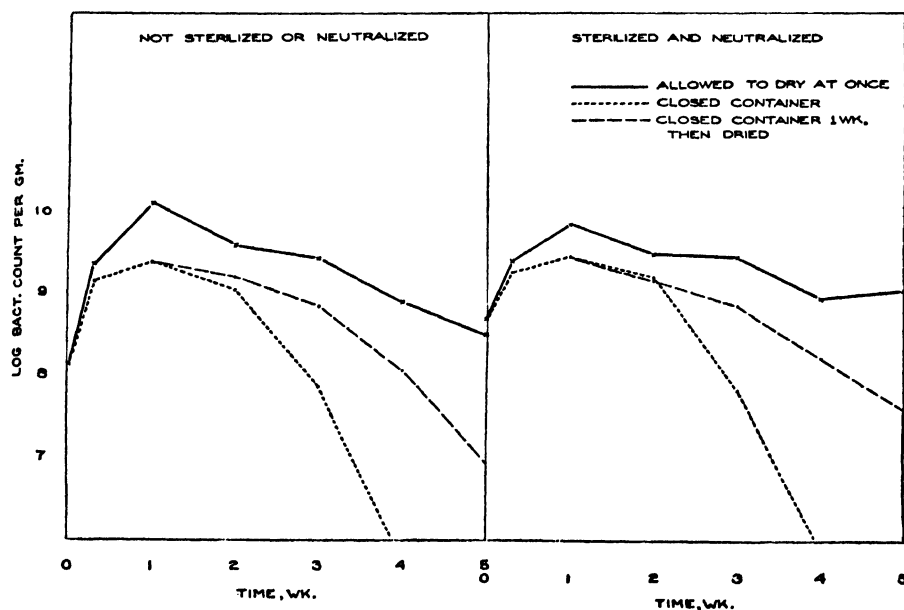


FIG. 4. Effect of desiccation on survival of *Salmonella typhimurium* on spongy peat pellets.

evidence suggesting that 'sterilization' may make the medium more suited to the growth of certain micro-organisms that may be present as heat-resistant types or contaminants. In an experiment in which total counts as well as *S. typhimurium* counts were made with sterilized and unsterilized pellets kept without special protection from contamination following inoculation, the spread was found to become wider with the sterilized pellets.

Certain inoculants for *Rhizobium* of the 'humus' type are not sterile prior to inoculation, but may contain large numbers of actinomycetes and spore-forming bacteria. Since these groups are known to include species that may be highly antagonistic to other bacteria, the presence of such forms may be a factor in determining the value of sterilization in a given case.

### Effect of Desiccation

To study more closely the effect of drying on the ability of spongy pellets to carry bacteria, comparative tests were made in which pellets were stored under different conditions:

- (i) Allowed to dry immediately after inoculation.
- (ii) Kept in closed containers.
- (iii) Kept in closed containers for one week, then allowed to dry.

The tests were carried out on two lots (*a*) not sterilized or neutralized, and (*b*) sterilized and neutralized prior to inoculation with *S. typhimurium*. The relevant data, including pH values and average pellet weights, are summarized in Table II while for comparison the viable bacteria counts, in terms of organisms per gram, are presented graphically in Fig. 4.

In both the 'unsterilized' and 'sterilized' series, results were similar in showing the advantage of allowing the spongy peat to dry immediately after inoculation. Not only were higher numbers of organisms per gram obtained (maximum  $1.2 \times 10^{10}$ ) but the counts were much better maintained during subsequent holding. Storage for one week in closed containers, followed by drying, was much less satisfactory for promoting longevity of the inoculated bacteria, though much superior to continuous storage in closed containers.

It is of interest to note the pronounced difference in the effect of immediate desiccation on the capacity of spongy peat to carry bacteria, as compared with that of powdered humified peat, as previously reported (1). In the latter case, immediate exposure to drying following inoculation did not show to the same advantage. With the powder it was necessary to prevent drying sufficiently to permit a preliminary increase in bacterial numbers. After this increase numbers were maintained at constantly higher levels than when the carrier was allowed to dry immediately.

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## QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

### VII. THE 'RHIZOSPHERE EFFECT' IN RELATION TO THE AMINO ACID NUTRITION OF BACTERIA<sup>1</sup>

By A. G. LOCHHEAD<sup>2</sup> AND R. H. THEXTON<sup>3</sup>

#### Abstract

Comparative studies of the relative incidence of bacteria of different nutritional requirements in soil indicate that one of the most characteristic rhizosphere effects is the preferential stimulation of bacteria requiring amino acids for maximum growth. Organisms for which amino acids are either essential or stimulative were proportionately increased in the rhizosphere. No similar effect was noted with respect to bacteria responding to growth factors.

The findings suggest, by indirect evidence, that the effect is to be ascribed to the excretion of amino acids by the growing plant. However, though this may be the chief factor, the preferential stimulation of the amino acid group of bacteria may be related to associative and antibiotic effects exerted by other bacteria, stimulated in the rhizosphere, observed to have different degrees of compatibility towards those responding respectively to amino acids and growth factors.

#### Introduction

It is now well established that the growing plant may exert a marked effect, both quantitative and qualitative, on the soil micro-organisms within the zone of influence of the root system (rhizosphere). Soil in the vicinity of the roots supports usually noticeably higher numbers of organisms than soil more distant from the plant, the effect varying with such factors as the kind of crop, the age of the plants, soil treatment, and moisture conditions (1, 3, 5, 11, 12, 14, 15). The rhizosphere of diseased plants may contain greater concentrations of micro-organisms than that of healthy plants (4, 13), while there is evidence that varieties susceptible to certain soil-borne diseases, even though healthy, may show higher numbers of organisms in the rhizosphere than corresponding resistant varieties (15). The 'rhizosphere effect' may be shown, not only in total numbers, but in a preferential stimulation of certain physiological groups of micro-organisms, as judged by quantitative determinations with selective media (2, 3, 5, 7, 8, 11, 12).

Earlier papers in this series (9, 17) have dealt with the influence of plant growth on the characteristics of soil bacteria studied by a quanti-qualitative procedure based on single culture isolations from a non-selective medium, chosen to permit the most nearly representative growth of the indigenous microflora. On the basis of morphology and taxonomic relationship, as well as physiological characters, Lochhead (9) found marked differences in the predominant bacteria in the rhizosphere as compared with soil distant from plant roots, similar trends being noted with all crops studied—clover, oats,

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mangels, corn, tobacco, and flax. In a study of the nutritional requirements of bacteria, West and Lochhead (17) showed that the relative incidence of bacteria for which amino acids and growth factors were essential or stimulative was higher in the rhizosphere of flax and tobacco plants than in corresponding control soils. Using a similar procedure, Katznelson and Richardson (6) found a higher proportion of bacteria stimulated by amino acids in the rhizosphere of tomato plants than in the control soil.

In the nutritional studies referred to, classification of the bacteria was made by observing relative growth in three media, consisting of a basal medium without addenda, and with the addition of amino acids or known growth factors. While these media permit a certain grouping of the organisms, a more complete differentiation, based on nutritional requirements, was provided by the procedure of Lochhead and Chase (10), which involved a determination of growth response in seven cultural media ranging from a simple basal medium to those containing amino acids, growth factors, amino acids with growth factors, or the unidentified substances present in yeast or soil extract or both. This report deals with the rhizosphere effect on the predominant soil bacteria classified on this basis.

### Experimental

Bacteria were isolated from control soil and from the rhizosphere of mangels from a four-year rotation system with oats, clover, and timothy. The soil had been maintained at good fertility level by regular application of farmyard manure. For the rhizosphere sample, soil adhering to the roots was used while the control soil was taken midway between rows. Samples were plated on a non-selective soil extract agar without added energy material (3), plates being held at 25° C. for two weeks. Colonies were systematically picked, approximately 180 for each sample, so that all on a plate or sector were taken, and stab inoculation made into soil extract semi-solid (containing 0.02% dipotassium hydrogen phosphate, 0.01% yeast extract, and 0.3% agar).

The nutritional grouping of the isolates was carried out by the procedure previously described (10) involving growth response in all of the following media:

- Medium *B* —Basal medium
- Medium *A* —Basal medium + amino acids
- Medium *G* —Basal medium + growth factors
- Medium *AG*—Basal medium + amino acids + growth factors
- Medium *Y* —Basal medium + yeast extract
- Medium *S* —Basal medium + soil extract
- Medium *YS*—Basal medium + yeast extract + soil extract

### Nutritional Groups in Rhizosphere and Control Soil

The relative incidence of the various nutritional groups of bacteria in the control and rhizosphere soils, and the estimated number of organisms of the different groups per gram of soil are shown in Table I. The outstanding



TABLE I

NUTRITIONAL GROUPS OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

Group	Nutritional requirements for maximum growth	Control soil		Rhizosphere soil		Times increase in rhizosphere
		%	Number per gm.	%	Number per gm.	
	(Plate count)		37,500,000		532,000,000	14.2
I	Grow in basal medium	12.0	4,500,000	22.5	119,700,000	26.6
II	Require one or more amino acids	6.8	2,550,000	25.0	133,000,000	52.2
III	Require growth factors	23.1	8,660,000	15.0	79,800,000	9.2
IV	Require amino acids and growth factors	16.2	6,080,000	15.0	79,800,000	13.1
V	Require yeast extract	16.2	6,080,000	11.7	62,200,000	10.2
VI	Require soil extract	6.8	2,550,000	5.8	30,800,000	12.1
VII	Require yeast extract and soil extract	11.1	4,160,000	2.5	13,300,000	3.2

feature is the increased percentage, in the rhizosphere, of organisms requiring amino acids for maximum growth (Group II), as compared with the control soil. This preferential stimulation is also reflected in the total numbers of this group in the rhizosphere soil, representing a 52-fold increase as compared with a 14-fold increase in total numbers. The table also shows that those groups of bacteria that are dependent upon the more complex nutrients provided by yeast extract and soil extract (Groups V, VI, VII) and not furnished by the amino acids and growth factors, are relatively less abundant in the rhizosphere. This is specially true of those organisms that require for maximum growth certain unidentified substances in both yeast and soil extract (Group VII). On the other hand, those forms that are capable of maximum growth in the basal sugar-salts medium (Group I) are relatively more abundant in the rhizosphere.

#### *Amino Acid Requirements*

The relation of amino acid nutrition to the growth of bacteria in the rhizosphere and control soils is presented in greater detail in Table II. A comparison of the growth of the bacteria in the seven media indicated above permits, not only a grouping on the basis of maximum growth, as in Table I, but also a determination of the incidence of those organisms for which amino acids act as essential substances or as a stimulant for growth. In addition to those from the mangels experiment (1945), Table II gives results from earlier rhizosphere studies,\* in so far as the relation to amino acids can be calculated from the data. In the earlier work all seven comparative media now employed were not used, but consisted in the 1941 series of Media B, A, and G, and in the 1939 tests of B, A, and AG; thus the same differentiation now possible could not be made.

\* Carried out with the co-operation of P. M. West (1939), and J. J. R. Campbell (1941).

TABLE II  
AMINO ACIDS IN NUTRITION OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

	Mangels (1945)		Flax (1941)		Flax (1939)		Tobacco (1939)	
	%		%		%		%	
	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.
No growth or submax. growth in basal med.								
Max. growth with amino acids	6.8	25.0	8.4	17.3	4.3	16.5	11.8	13.6
Max. growth, cysteine alone	4.3	13.3	3.1	5.8	—	—	—	—
No growth in basal medium								
Growth (max. or submax.) with amino acids	20.5	30.0	7.3	11.0	14.9	29.1	14.0	24.0
Growth (max. or submax.) with cysteine	13.7	20.9	7.3	10.5	—	—	—	—
Submax. growth in basal medium								
Stimulated by amino acids	10.2	13.3	4.3	14.1	0	14.8	9.6	13.1
Stimulated by cysteine alone	6.0	8.3	4.3	8.8	—	—	—	—
Submax. growth in Medium G								
Stimulated by amino acids	17.9	20.8	—	—	—	—	—	—

From Table II it is seen that in all cases there is a preferential stimulation in the rhizosphere of those bacteria for which amino acids provide maximum growth, are essential for growth, or stimulate growth. This is believed to be one of the most characteristic rhizosphere effects as far as the nutritional characteristics of soil bacteria are concerned.

#### *Growth Factor Requirements*

Analogous data with respect to the growth factor nutrition of bacteria in rhizosphere and control soils are shown in Table III with the inclusion of

TABLE III  
GROWTH FACTORS IN NUTRITION OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

	Mangels (1945)		Flax (1941)		Flax (1939)		Tobacco (1939)	
	%		%		%		%	
	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.
No growth or submax. growth in Med. B or A								
Max. growth with growth factors	23.1	15.0	23.2	17.8	8.6	12.4	19.3	19.3
Max. growth, growth fact. and amino acids	16.2	15.0	—	—				
No growth in Med. B or A								
Growth (max. or submax.) with gr. factors	15.4	19.2	13.7	7.9	14.9	11.0	17.2	15.6
Growth (max. or submax.) with gr. factors and amino acids	7.7	5.0	—	—				
Submax. growth in Medium B								
Stimulated by growth factors	9.4	3.3	7.3	8.4	—	—	—	—
Submax. growth in Medium A								
Stimulated by growth factors	20.5	10.0	—	—	4.3	16.7	20.4	26.1

findings from earlier studies as far as the data permit calculation. In contrast to the results for amino acids, no significant 'rhizosphere effect' is noted with respect to the relative incidence of bacteria responding to the growth factors used.

### Associative and Antagonistic Effects

Though the equilibrium between various groups of organisms existing in a soil at a given time will depend in large measure upon the availability of nutrients required for the growth of these organisms, associative and antagonistic effects are factors that might well play a role in establishing the microbial balance under a given set of conditions.

As noted in Table I, bacteria of Group I, capable of maximum growth in the basal medium, were relatively more numerous in the rhizosphere than in the control soil, though stimulated less than organisms of Group II. An attempt was made to study the action of Group I organisms on those of Groups II and III, respectively, by noting the effect of the culture filtrates of the first group on the growth of the other groups: (a) in the basal medium in which they normally do not grow, and (b) in Media A and G, respectively, to observe antibiotic effect in media in which they normally grow well.

Ten organisms of Group I were inoculated respectively into 50 ml. of the basal medium (B). After five days' incubation at 25° C. the cultures were filtered (Seitz) and 0.5 ml. of the respective filtrates added aseptically to different series of tubes each containing 5 ml. of Medium B or Medium A. Tubes containing filtrate from each of the Group I organisms, as well as control tubes without filtrate, were inoculated with one 1-mm. loopful of a suspension of each of 10 different strains of Group II organisms in respective series and incubated at 25° C. for five days. The tubes were then examined to note the effect of the different filtrates on the growth of each of the test organisms of Group II. Analogous series were prepared with Medium B and Medium G to note the effect of similar filtrates on Group III organisms.

The results, shown in Table IV, indicate that as far as the organisms tested are concerned, bacteria of Group I not only provide much greater stimulation to those of Group II than Group III, but are much more antagonistic towards Group III. This greater compatibility between Groups I and II than between Groups I and III is shown by a higher percentage of cases in which the filtrates permit Group II bacteria to grow in the basal medium (46.7% as compared with 18.2%), and by a much lower percentage of cases in which antibiotic effects were noted (4.4% as compared with 38.7%).

### Discussion

The increased incidence in the rhizosphere of bacteria capable of maximum development in an amino acid medium or stimulated by amino acids raises an interesting speculation as to the relation of this to plant excretions, the exact nature of which is little understood. Though amino acids have been detected in soil and are regarded chiefly as products of the decomposition of organic

PLATE I

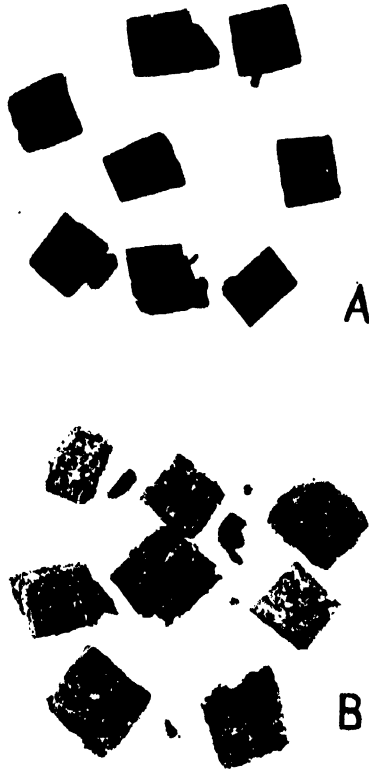


FIG. 1. Peat pellets: A, hard pellets, prepared from compressed, humified peat; B, soft pellets, from spongy peat.



nitrogenous residues, the excretion of amino acids by plants was established by Virtanen and Laine (16) who identified aspartic acid and  $\beta$ -alanine in the nitrogenous excretions of leguminous plants. The environmental factors determining such excretion have been thoroughly investigated by Wilson and associates (18). That amino acids may be excreted by non-leguminous plants in amounts sufficient to modify the balance of nutritional groups of bacteria may be postulated.

The formation of amino acids as decomposition products of sloughed-off portions of roots also suggests itself as a possible factor in the rhizosphere effect on the amino acid group of bacteria. Excretion by the root appears more likely, however, inasmuch as the effect may be exerted by the plant in the seedling stage, as was the case in the 1939 and 1941 experiments with flax (Table II).

TABLE IV

ASSOCIATIVE AND ANTAGONISTIC ACTION OF GROUP I ORGANISMS ON BACTERIA REQUIRING AMINO ACIDS AND GROWTH FACTORS (GROUPS II AND III)

Filtrates of Group I bacteria* (Cult. No.)	Group II bacteria**				Group III bacteria***			
	No. of strains tested	No. able to grow in basal med. + filtrate	No. suppressed by filtrate in Med. A		No. of strains tested	No. able to grow in basal med. + filtrate	No. suppressed by filtrate in Med. G	
			Partially	Completely			Partially	Completely
C77	10	3	2	0	11	0	4	0
R10	—	—	—	—	11	5	3	1
R14	10	4	1	0	11	1	3	4
R34	10	2	1	0	—	—	—	—
R36	10	3	0	0	11	1	3	1
R52	10	8	0	0	11	0	4	2
R85	10	5	0	0	11	3	4	0
R86	10	6	0	0	11	1	3	2
R87	10	6	0	0	—	—	—	—
R115	10	5	0	0	11	5	0	0
		%	%	%		%	%	%
		46.7	4.4	0		18.2	27.3	11.4
			4.4				38.7	

\* Maximum growth in basal medium.

\*\* No growth in basal medium; require amino acids.

\*\*\* No growth in basal medium; require growth factors.

The data in Table IV suggest that the preferential stimulation of the amino acid group may be concerned, at least partially, with the phenomenon of associative action between different groups of organisms. This influence, however, may be related indirectly to a shifting of the bacterial equilibrium due to plant excretion, for instance, through a stimulation of Group I bacteria, which might in turn stimulate Group II.

The data here presented furnish indirect rather than direct evidence regarding the mechanism by which the development of special groups of soil bacteria

in the rhizosphere is induced. However, the study of nutritive differences in micro-organisms in the rhizosphere is offered as one useful means of approach in obtaining a better knowledge of the physiological activity of the root system of plants, and of factors concerned with such practical problems as crop rotation and the control of soil-borne diseases of crops.

### Acknowledgment

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# FACTORS INFLUENCING GROWTH AND SUMMER DORMANCY IN *TARAXACUM KOK-SAGHYZ*<sup>1</sup>

BY G. W. SCARTH,<sup>2</sup> H. B. GOODING,<sup>3</sup> AND M. SHAW<sup>3</sup>

## Abstract

A setback in rosette diameter and leaf number was observed in both first and second year *Taraxacum kok-saghyz* plants at a definite stage in the plant ontogeny whatever the time of planting. This condition was evidently not entirely synonymous with the 'summer dormancy' of Russian workers as it did not involve complete loss of leaves, nor endure so long. The length of the 'dormant' period, which occurred at the time of reproduction and could be delayed, although not prevented by removal of flower buds, varied from about two to eight weeks. Various external modifications of environment had no appreciable effect on its onset. Watering and disbudding each increased the total fresh weight yield. Watering affected rosette diameter more markedly than it did root weight; disbudding affected both to approximately the same extent. Shading was not beneficial to growth.

The phenomenon of 'summer dormancy' in *Taraxacum kok-saghyz* is reported in Russian literature to be of the greatest importance in reducing the potential yield of the crop, but the writers do not agree as to the extent to which its incidence can be avoided, especially in the second and subsequent years of growth. Some, e.g., Neiman and Sosnovets (4) believe that the dormancy is entirely dependent on climate; others, such as Lebedeva (2), that it is an unavoidable phase of development. Tikhovskaia (5), from a survey of the literature, concludes that *T. kok-saghyz* and also *T. tau-saghyz* have an inherent tendency to enter a rest period at a definite stage of their ontogeny, but that the time of onset and duration of the rest period are modified by external conditions.

Experience in cultivation of *kok-saghyz* in North America indicates that, despite the high quality of *kok-saghyz* rubber, the crop must give greatly increased yields if it is to be of any economic importance. Accordingly, the present experiment was designed to repeat a study of the factors that are said to reduce dormancy, estimating their effect, not only from ultimate yield, but also from the progress of growth during the critical summer period.

## Plan and Method

The experiment was laid down in the Macdonald College Department of Horticulture on a plot provided with a sprinkler system for watering. The soil is a sandy loam.

In order to distinguish internal rhythm from the effects of seasonal change, weather conditions, and other environmental differences, rows were planted

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<sup>2</sup> At the time, Professor of Botany.

<sup>3</sup> At the time, senior student in Botany.



at different times. Several rows were carried over from the previous year, and various sowings were made from late March to late June of the current year (1944).

Experimental modifications of environment consisted of (1) watering, (2) shading by muslin screens, and (3) removal of flower buds as formed. The watering was applied to half of the plot, that is, to half of all plantings, the other treatments to portions of rows including second year plants and two early sowings of first year plants. Watering was done for an hour each day, except in very dull weather, from the end of May until the beginning of September, the object being to reduce sun-heating of the soil as well as to supply additional water to the plants. The other treatments were applied to both unwatered and watered sections. Shading of second year plants was begun on June 28. Removal of flower buds (hereafter termed disbudding) was started at the beginning of flowering at the end of May and continued until the end of the reproductive period. In addition to notes on development, growth was estimated quantitatively by counting the number of leaves in several rosettes of certain plantings and measuring the diameters of the same and other plants at intervals throughout most of the growing season.

In order to determine the effects on yield, samples representing the various plantings and treatments were harvested on Sept. 21 and 22 and again on Oct. 21. The plants were uprooted carefully with a long spade to avoid severe damage to the root systems. The tops were severed from their roots by a transverse cut just below the crown, each was washed free of soil, air-dried for 5 to 10 min. and then weighed. The roots were dried in a soil sterilizer at 55° to 60° C. until quite brittle and weighed again.

Cultural procedure common to the whole plot included fall ploughing and manuring, watering as required until seedlings appeared or until transplants were established, application of liquid fertilizer on May 31, except to the later sowings, and on June 19 to all the rows, and cultivation and hand picking to keep down weeds. The rows were 18 in. apart, transplants were set 10 in. apart in the rows, and field sowings were thinned with the hoe to small groups of plants at about 6 in. intervals. A variation, which was not part of the original design, was introduced. The earlier field sowings, made at a depth of 1 to 1.5 cm., gave slow and irregular germination, probably due in large measure to the formation of soil crust. Consequently, in later sowings, changes were made to accelerate germination.

Table I summarizes the different plantings and their establishment and survival. Three different seed samples *A*, *B*, and *C* were used, all provided by the Division of Botany, Central Experimental Farm, Ottawa. The range of forms among the plants grown from the different samples did not differ significantly. About 10% were weed species, and these were mostly eliminated in transplanting or thinning.

The table shows that transplantation of seedlings started in the greenhouse was very successful, aided, no doubt, by early watering. Field sowing, at least in a soil that tends to become crusted, requires to be shallow, about

TABLE I

TIMES AND MODES OF PLANTING VS ESTABLISHING OF PLANTS

Year	Transplants				Field sowings				
	Seed sample	Sown in greenhouse	Transplanted	Established	Seed sample	Sown	Depth, cm.	Appearance	Stand
1943	One row A	April	May	90%	Several rows	May	1-15	Slow	Very sparse
1944	One row B	March	May 9	90%	Two rows B	May 12	1-15	18 days	Poor and irregular
	One row B	April	May 29	90%	Two rows B	June 2 (vernalized)	1-15	11 days	Fairly regular
					One row C	June 12	0.5	6 days	Excellent
					One row C	June 22	0.5	6 days	Excellent

0.5 cm. Deeper sowing was improved somewhat by vernalization of the seed (soaking for three weeks at 1° to 2° C.). No pretreatment could much improve the stand obtained with untreated seed, when sown at a depth of 0.5 cm. or less in well prepared soil and kept moist until the plants appeared.

Plantings of the previous season suffered little frost killing during the winter, but showed great mortality from root rot during their second summer. Injury began to show about May 15; about 30% of one row was dead by the end of June, and 42% by Sept. 25. The rot appeared to be most common where plants were crowded, this fact suggesting a spread of infection from plant to plant.

## Results

### A. INTERNAL CYCLE OF THE PLANT (FIG. 1)

#### I. GENERAL BEHAVIOUR

All the plantings showed a definite developmental cycle, which, within the limits of the respective growing periods, was adhered to regardless of the time of planting. Early growth was characterized by a rapid increase in size as measured by rosette diameter and leaf number. Flowering began shortly before the attainment of maximum size and the period between the peak and completion of flowering marked the beginning of a decline in size. This period of setback will be referred to provisionally as the dormant period. Its relation to 'summer dormancy' referred to in the Russian literature will be discussed later. The dormant period, which varied in length from about two to eight weeks was followed by a period of renewed, though perhaps less intense, activity during which leaf number and rosette diameter rose again fairly sharply. As before, this vegetative growth terminated in a reproductive phase, and again a decrease in size set in before the end of flowering.

While the pattern of the cycle is the same for first and second year plants there are, however, certain apparent differences. The second year plants reached their maximum size by mid June, and flowered continuously from

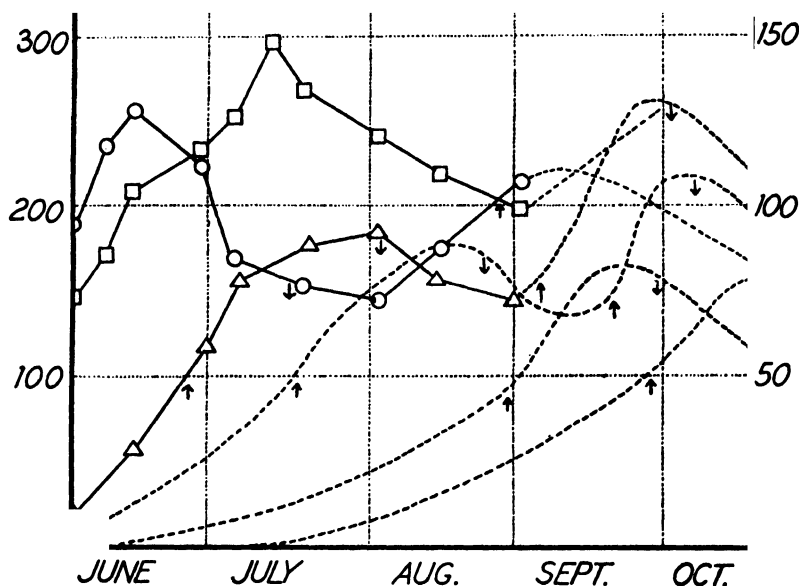


FIG. 1. Comparison of growth cycles of different plantings. Continuous lines represent actual counts of leaf number plotted from the average value of several plantings: broken lines are hypothetical curves constructed from general observations of May 29 transplants and of the averages of May 12 and June 2 and of June 12 and 22 plantings respectively. The scale for first year is double that for second year plantings. ○ = second year. □ = second year disbudded. △ = first year May 9 transplants. ↑ = beginning and ↓ = end of flowering.

May 15 until July 15. Yellowing of the outer leaves was observed as early as May 22 but the decline in leaf number, which lasted for eight weeks and cut the rosettes to one half or less than one half their diameter, began at the height of flowering in mid June. In the first year plants on the other hand, though yellowing of the leaves was noted at the beginning of flowering, the decline in leaf number and rosette diameter did not start until the end of the flowering period, which lasted four to five weeks, e.g., in the earliest planting from the end of June until about Aug. 1. This decline continued only for two or three weeks and did not compare in intensity or in duration with the setback shown earlier in the season by the second year plants. In both first and second year plants the second period of vegetative growth and flowering was less profuse than the first.

As regards root growth the harvesting yields show a decrease in the fresh weight of roots as well as tops between Sept. 21 and Oct. 21 in those plantings that were completing a period of flowering at that time. The decrease of the roots is proportionately much smaller than that of the tops. Thus during dormancy the top/root ratio declines and a low ratio may be taken as a sign of dormancy. Table II shows differences of behaviour as regards growth

TABLE II

FRESH WEIGHT IN GRAMS PER PLANT AND TOP/ROOT RATIO (T/R) AT HARVESTING

Date of harvesting		Sown:					
		Mar.	Apr.	May 12	June 2	June 16	June 22
Sept. 21	Av. wt. T/R	74 1.1	52.5 1.35	41 2.4	33 1.85	26.5 1.8	16 2.15
Oct. 21	Av. wt. T/R	69 1.0	50.5 1.13	39 1.45	33 1.55	28.5 1.6	17.3 1.25
Wt. change, % Sept. 21 to Oct. 21		-7	-4	-5	0	+8	+9

between Sept. 21 and Oct. 21 depending on the age of the plants, as discussed in the next paragraph.

## II. RELATION TO TIME OF PLANTING

The earliest sown first year plants, which were transplanted to the field on May 9, passed through their first dormant period during the first two or three weeks of August, and began their second decline early in October. Root growth ceased after Sept. 21.

The second set of transplants, set out about three weeks later, first showed dormancy during the last week of August and the first part of September. Their root yields on Sept. 21, as compared with those of the first transplants, as well as their higher top/root ratios suggest that they had not completed their second grand period by that time, although by Oct. 21 a decline in top weight seemed to have begun.

The field sowings of vernalized seed made on May 12 and June 2 were noted as beginning to flower about Aug. 30, the average time after germination being about the same as with those started earlier in the greenhouse. Between Sept. 21 and Oct. 21 these sowings showed little change in total weight and a marked decline in top/root ratio, which, in conjunction with their date of flowering, indicates that, flowering having been completed, their rosettes were passing through their first decline in October.

The latest sowings (June 12 and 22) required all that was left of the growing season to pass through the first grand period of their growth. The average diameter of the rosettes of the June 22 sowing increased from 5 in. on Aug. 15 to 7 in. on Sept. 9, the increase continuing through September. At the end of September the plants were just beginning to flower and the weight of the plants increased considerably between Sept. 21 and Oct. 21, as the harvesting results indicate.

Fig. 1 summarizes the growth cycles of the different plantings in graphical form. Only the continuous lines represent actual counts of leaf number. The remainder indicate probable trends as inferred from notes on development,

from diameter measurements of the two latest sowings, and from the fresh weights of the tops at the different harvestings.

In a growing period of seven months the second year plants completed, and first year plants almost completed, two growth cycles. With later sowings and shorter growing periods the development was terminated at progressively earlier stages, but as far as it went showed no essential difference. Since in the different plantings decline occurred in June, July, August, September, or October depending on the age of the plants this is not simply a summer phenomenon. Dormancy tends to occur at a definite stage of ontogeny, but is far more severe in second than in first year plants.

### III. INDIVIDUAL CYCLES (FIG. 2, TABLES III AND IV)

In Fig. 1, in which the curves are plotted from the average values for several plants, the dormant period appears to be extended over a longer period of time and to be accompanied by less of a decrease in growth and size than was actually the case. This is due to slight differences between the individual plants.

TABLE III

LEAF NUMBER AND ROSETTE DIAMETER (IN INCHES) OF FIRST YEAR SAMPLE PLANTS

Treatment	Rosette measurements	June				July		August			September
		2	9	16	30	7	21	1	15	29	18
Watered	Leaf No.	15	21	38	90	147	190	198	135	105	196
	Diam.	6½	8	11	15	15	14	14	12	7	
	No.	10	15	23	40	53	58	54	41	60	65
	Diam.	4	5	6	8	9	8	6	7	8	
	No.	16	20	26	86	83	90	91	89	130	214
	Diam.	3	3	4½	7	8	9	8	7	8	
	No.	16	24	33	55	74	66	74	51	55	60
	Diam.	6	7	10	12	13	13	12	5	4	
Unwatered	No.	15	21	33	62	87	110	103	86	118	150
	Diam.	5	6	9	11	12	11	9	10	10	
	No.	19	24	30	55	84	102	118	125	121	163
	Diam.	6	9	10	13	14	13	13	12	11	
	No.	18	29	44	88	141	155	120	160	90	100
	Diam.	5	6	6	8	9	9	10	4	3	
	No.	12	17	24	28	48	58	71	67	75	68
	Diam.	4	5	6	9	9	9	9	7	8	
Unwatered, shaded	No.	17	17	36	55	74	67	83	50	36	93
	Diam.	5	6	7	9	10	8	8	2½	3	
	No.	10	13	23	33	44	42	41	33	35	51
	Diam.	4	5	6½	7	9	9	6	5	4	
	No.					77	69	69	69		55
	Diam.					10	12	10	7		
	No.					99	110	165	191		290
	Diam.					12	11	11	10		
	No.					84	98	107	169		205
	Diam.					12	12	11	11		
	No.					68	67	66	71		70
	Diam.					11	11	9	7		
	No.					78	68	75	66		64
	Diam.					7	10	7	6		

TABLE IV

LEAF NUMBER AND ROSETTE DIAMETER (IN INCHES) OF SECOND YEAR SAMPLE PLANTS

Treatment	Rosette measurements	May	June				July			August		
		31	7	14	28	5	12	19	2	16	31	
Unwatered	Leaf No.	75	116	136	173	182	181	205	163	110	95	
	Diam.	9	12	12	14	12	13	12	10	8½	9	
	No.	240	275	330	246	186	85	54	50	95	120	
	Diam.	16	16	16	16	12	12	9	3	2	3	
	No.	170	220	210	115	80	27	19	23	30	40	
	Diam.	15	15	16	15	12	10	9	1	3	4	
Watered	No.	179	231	263	208	157	194	208	216	323	374	
	Diam.	13	14	13	14	14	12	9	11	10	12	
	No.	254	311	304	273	231	268	244	238	265	345	
	Diam.	12	13	13	13	13	12	12	9	8	8	
Disbudded, unwatered	No.	205	230	220	195	235	260	260	305	290	200	
	Diam.	11	11	12	10	10	10	12	12	10	10	
Disbudded, watered	No.	118	166	215	324	375	425	391	354	328	340	
	Diam.	12	13	14	14	14	14	13	13	10	9½	
	No.	219	289	329	375	400	495	520	341	332	316	
	Diam.	13	14	15	15	16	16	16	15	12	11	

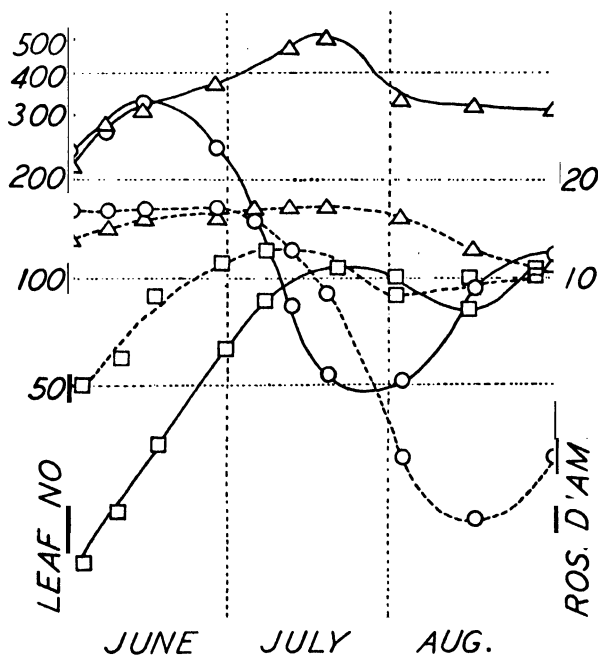


FIG. 2. Comparison of leaf number and rosette diameter of individual plants. Continuous line = leaf number. Broken line = rosette diameter.  $\square$  = first year.  $\circ$  = second year.  $\triangle$  = second year disbudded.

Fig. 2, in which the curves are for typical individual first and second year plants, probably presents a truer picture of the duration and degree of dormancy. From Fig. 2 it is seen that in first year plants reduction in rosette diameter precedes reduction in leaf number by approximately two weeks, whereas in second year plants the reverse is true, and reduction in leaf number occurs approximately two weeks before reduction in rosette diameter. Disbudded second year plants are intermediate in their behaviour; reduction in leaf number is coincidental with reduction in rosette diameter. The relationship between these three types of cycles and its bearing on the nature of dormancy is discussed further below.

## B. EFFECT OF EXTERNAL FACTORS ON THE GROWTH CYCLE

### I. WEATHER CONDITIONS

The weather from May until September 1944 was characterized by a high proportion of bright hot days and by a low over-all rainfall. Particularly

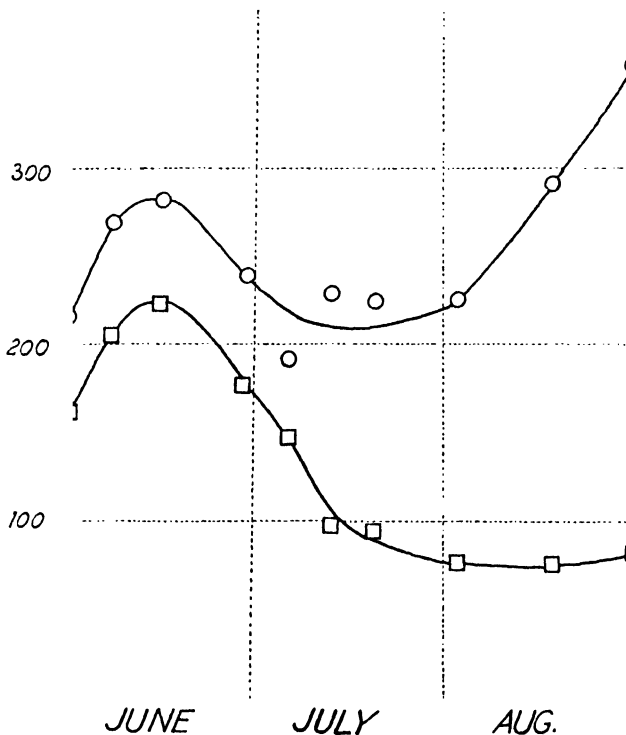


FIG. 3. Average variation in leaf number of watered and unwatered second year plants.  
 □ = unwatered. ○ = watered.

unfavourable conditions prevailed from June 26 until Aug. 17, during which period the temperature and light intensity maintained very high values. If dormancy were induced by unfavourable environmental conditions regardless

of the developmental stage of the plant, all the plants in the experimental plot should have been dormant during this 10 wk. period, as were the earliest plantings; such, however, was not the case. Second year plants entered on a new period of vegetative growth in the middle of the hot spell and those plants grown from seed sown on June 2, 12, and 22 increased steadily in rosette diameter and leaf number from the time of planting until mid August, September, and October respectively. It is evident that while unfavourable conditions of environment may slow the rate of growth, they cannot bring about the changes characteristic of dormancy.

## II. WATERING

Fig. 3 shows the average variations in leaf number of two watered and three unwatered second year plants during the period June to August. Fig. 4 shows the variation in rosette diameters of first and second year plants during the same period. Tables III and IV present data for leaf number and rosette diameter of first and second year plants.

Watering in no way affected the essential pattern of the developmental cycle and did not prevent dormancy, though the decrease in size may be some-

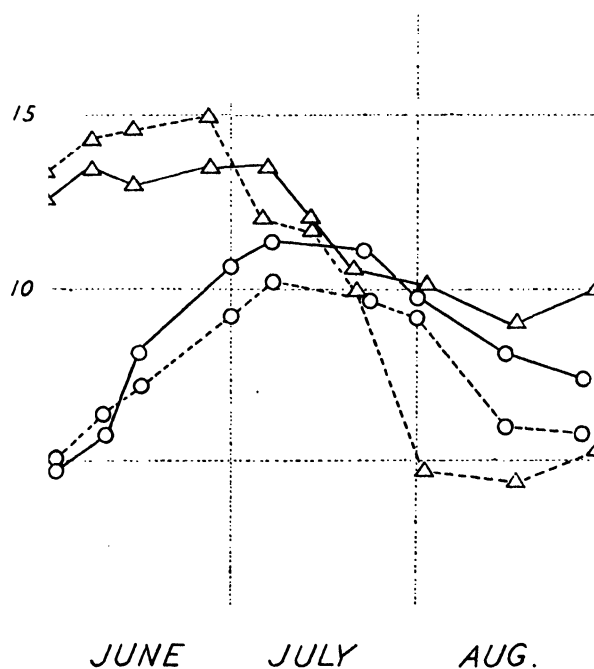


FIG. 4. Average variation in rosette diameter of first and second year plants. ○ = first year. △ = second year. Continuous line = watered. Broken line = unwatered.

what reduced. The main effect was to increase the size of the plants. This is further discussed under yields. Watering may delay the onset of dormancy; the results are not, however, significant.



## III. SHADING (TABLE III)

Shading with strips of light factory cotton about 40 ft. long and 3 ft. wide was started in the watered and unwatered second year plots on June 9 and in the first year plots on June 28. The majority of the second year plants under observation were killed by a root rot of an unascertained nature. Observation, however, indicated that while shading may to some extent prevent or lessen the midday wilting, which is particularly noticeable in those plants that have just completed flowering, it does not in any way prevent dormancy in either first or second year plants.

## IV. DISBUDDING (TABLE IV)

The flower buds were removed from a number of second year plants as soon as they formed. Several of the plants were killed by root rot, but the results show definitely that, though dormancy was not prevented, the onset of the dormant period was delayed by about three weeks. Fig. 1 shows the average variation in leaf number of four disbudded plants. These results confirm those of Lebedeva (2). Disbudding is discussed in relation to yield below.

TABLE V

YIELDS OF FIRST YEAR *Taraxacum kok-saghyz* SAMPLES HARVESTED ON SEPT. 21 AND OCT. 21

Sample	Date of field planting	Pretreatment	Field treatment	September				October			
				Fresh weight in gm. per plant			Top: root ratio	Fresh weight in gm. per plant			Top: root ratio
				Total	Top	Root		Total	Top	Root	
40 B plants	May 9	Transplants sown in greenhouse end of March	Unwatered	62.0	28.6	33.4	0.85	54.1	22.1	30.0	0.69
			Watered	86.2	50.9	35.3	1.40	85.7	48.0	37.7	1.3
40 B plants	May 29	Transplants sown in greenhouse mid April	Unwatered	57.6	32.5	25.1	1.3	59.2	30.5	28.7	1.06
			Watered	47.5	28.3	19.2	1.46	41.8	22.8	19.0	1.2
40 B plants	May 12	Untreated seed sown at 1 to 1.5 cm.	Unwatered	32.7	21.0	11.7	1.8	36.6	22.0	14.6	1.5
			Watered	51.1	38.5	12.6	3.05	40.7	23.7	17.0	1.4
50 ft. B row, various nos. of plants (40 to 65)	June 2	Vernalized, sown at 1 to 1.5 cm.	Unwatered	31.7	20.5	11.2	1.8	27.5	16.9	10.6	1.6
			Watered	34.0	22.5	11.5	1.9	40.2	24.3	15.9	1.5
				In gm. per foot of row				In gm. per foot of row			
50 ft. C row	June 14	Untreated, sown at 0.5 cm.	Unwatered	51.3	33.8	17.5	1.93	53.0	30.9	22.1	1.4
			Watered	54.5	34.0	20.5	1.66	62.5	40.0	22.5	1.78
50 ft. C row	June 22	Untreated, sown at 0.5 cm.	Unwatered	24.7	15.6	9.1	1.71	26.3	13.1	13.2	0.995
			Watered	40.0	29.0	11.0	2.64	40.5	24.5	16.0	1.53

From the foregoing it may be concluded that the developmental cycle is due to an internal rhythm that is independent of the environment. The latter cannot change the essential pattern but may exert some modifying effects. Though related in time to the period of flowering it is only postponed and modified by continuous removal of flower buds.

### C. YIELDS

Tables V, VI, and VII summarize the yields of sample plants representing the various experiments. Here the yields are expressed in grams per plant on a fresh weight basis, with the exception of the *C* plantings in which, as the plants were thinned in bunches at 6 in. intervals, they are in grams per foot of row.

#### I. RELATION TO TREATMENT

##### Total Yield

##### 1. Watering

As far as total weight was concerned, watering was found to be beneficial in all first year plants with one exception (Table V). In the May 29 transplants both the September and October yields of unwatered samples were

TABLE VI

YIELD OF FIRST YEAR SAMPLES HARVESTED SEPT. 21. THE WEIGHTS ARE AVERAGE FOR 40 PLANTS TAKEN FROM EACH GROUP

	Field treatment	Fresh weights in gm. per plant			Top: root ratio
		Total	Top	Root	
Sown in greenhouse end of March. Transplanted May 9	Unwatered	62.0	28.6	33.4	0.85
	Unwatered, shaded	55.0	24.6	30.4	0.8
	Watered	86.2	50.9	35.3	1.4
	Watered, shaded	83.2	53.0	30.2	1.8

TABLE VII

YIELDS OF SECOND YEAR SAMPLES HARVESTED SEPT. 22

Pretreatment	Sample	Field treatment	Fresh weights in gm. per plant			Top: root ratio
			Total	Top	Root	
Transplants sown in greenhouse March 1943	40 ft. row (=47 plants)	Unwatered	68.5	38.0	30.5	1.3
	40 ft. row (=33 plants)	Watered	64.0	43.5	20.5	2.2
	11 ft. row (=12 plants)	Unwatered, disbudded	86.0	50.0	36.0	1.3
	10 ft. row (=13 plants)	Watered, disbudded	90.0	62.0	28.0	2.2

higher than the corresponding watered ones. No explanation is offered for this discrepancy. Little effect, as a result of watering, was observed in the total weight of second year transplants (Table VII).

### 2. *Shading*

Data for the effects of shading are presented for a single planting in Table VI. Here shading was found to have a slightly deleterious effect on the growth of May 9 transplants. Confirmatory evidence was obtained from general observations of second year transplants.

### 3. *Disbudding*

A very marked increase in weight was observed as a result of disbudding of second year plants (Table VII). This greater weight of disbudded plants was undoubtedly due to the longer vegetative period already discussed under factors affecting the cycle and to the prevention of flower and fruit development and the consequent diversion of nutrient material to the vegetative organs.

### *Root Yield*

As most of the rubber is stored in the root the effects of treatment on the root yield are more important from the practical standpoint.

#### 1. *Watering*

Tables V, VI, and VII show that almost invariably the top to root ratio was higher in the watered than in the unwatered part of the plot. This indicates that watering has a more marked effect on the development of the shoot than of the root. However, in first year plantings the root weight was, with the exception of the May 29 transplants, higher in the watered than in the unwatered. The difference was never very large but appeared to be greater in the later sowings than in early ones suggesting that unwatered plants in the later sowings had greater difficulty in establishing their root systems than did early plantings, the roots of which had penetrated deeply before the onset of drier conditions in July and August. The possibility of the greater weight of watered roots being due to a greater succulence was eliminated by a comparison of fresh and dry weight. The fresh to dry weight ratio of a number of samples is presented in Table VIII. Slight fluctuations are probably due to incomplete drying.

Watering definitely retarded root growth of second year plants. It appears likely that this was due to poor aeration of the soil in the watered part of the plot for the soil had not been ploughed for two years and its upper layers often became waterlogged when watered.

#### 2. *Shading*

Root development, as was total development, was hindered by shading. The data are summarized in Table VI.

### 3. Disbudding

Removal of flower buds, as might be expected from a previous discussion, favoured root development. Table VII shows that the increase in weight of roots was proportional to that of tops.

TABLE VIII  
RATIO OF FRESH WEIGHT TO DRY WEIGHT OF ROOTS OF FIRST  
YEAR SAMPLES

Date of planting	Field treatment	Fresh weight/dry weight
May 9	Unwatered	4.22
	Watered	4.42
May 29	Unwatered	4.40
	Watered	4.32
May 12	Unwatered	4.0
	Watered	4.12
June 2	Unwatered	4.13
	Watered	4.04

## II. RELATION TO TIME OF PLANTING

Some indication of the importance of early planting on yield is presented in Table IX, where the estimates of root yields in kilograms per acre, on a dry weight basis, of samples of several plantings that were harvested on Oct. 21 are presented. Plants from seedlings that had been germinated in the spring

TABLE IX  
DRY WEIGHT ROOT YIELDS OF FIRST YEAR PLANTINGS HARVESTED OCT. 21 FROM UNWATERED  
PARTS OF THE PLOT

Date of field planting	Pretreatment	Dry weight of roots, kgm. per acre	
May 9	Sown in greenhouse end of March	257	Av.
			243
May 29	Sown in greenhouse mid April	230	
June 14	Untreated, sown at 0.5 cm.	153	123
June 22	Untreated, sown at 0-5 cm.	93	

in the greenhouse and transplanted later when climatic conditions were favourable gave average yields approximately twice as large as the June plantings. The ratio of the respective lengths of the growing seasons was considerably lower, namely, about 1.65 to 1.0, which reflects the relatively favourable conditions for photosynthesis of kok-saghyz during early summer, reflected also in the steepness of the curves of rosette growth during that period.

## Discussion

It has been concluded that in kok-saghyz a recession in rosette size occurs at the end of each grand period of growth. Root weight seems to share to some extent in the decline. While this phenomenon normally begins after the height of flowering, it is not eliminated by removal of flower buds and is therefore better described as the maturation than the reproductive phase of development. External conditions influence the degree and duration of the setback but neither induce nor prevent its occurrence. Though the condition has been referred to as dormancy in the foregoing description, the point at which growth actually ceases is not shown by the type of measurements made in these experiments, and moreover the condition is evidently not entirely synonymous with 'summer dormancy' as described in the Russian papers. The latter involves 'loss of leaves', presumably complete, and is said to begin about mid July and to last one and a half to two months. Under our conditions only a small minority of the plants, all of the second year, suffered to this extent.

Before commenting on the causes contributing to the extreme condition known as summer dormancy, let us consider the interaction of internal and external factors at the very beginning of the decline as revealed by analysis of some of our own results. The order of initiation of the decrease in leaf number and rosette diameter respectively seems to throw some light on the relationship. Second year plants passing into dormancy during the relatively cool weather of June show a marked decrease in leaf number about two weeks before rosette diameter begins to be reduced. This would seem to indicate that the setback begins with a reduction in the activity of the growing point. Similarly the later revival in growth shows an increase in leaf number about two weeks before rosette diameter. On the other hand, in those plants in which the setback occurs during the hot dry spell of July and early August the decline in rosette diameter begins relatively earlier. Thus in the second year plants that had their growth cycle prolonged until this period as a result of disbudding, the decline in rosette diameter begins simultaneously with that in leaf number, while in first year plants it precedes the latter considerably. So also, first year plants protected by shading during the hot weather, if they show any decline at all, follow the same sequence as do the second year plants during the cooler weather. In view of the severe wilting of the outer leaves of plants in the maturation phase during the unfavourable weather, it would seem that their earlier senescence and death was largely caused by an upset in water balance although the high temperatures may also have produced some direct injury. While the immediate cause of the increased death rate of the leaves is thus traceable in part to external factors, the sensitivity to these is due to internal changes since plants in the vegetative phase of development continued to increase in rosette diameter as well as leaf number throughout the testing period.

The greater tendency to loss of leaves in second year plants must also be attributed to internal factors, since in our experiments it was displayed

while the weather was still favourable for growth. These plants, which all flowered heavily, showed a greater percentage reduction of rosettes than flowering first year ones. A qualitative developmental difference between first and second year plants is the 'collar' formation or sloughing off of the old phloem in the roots of the latter. It appears unlikely that there is direct causal connection between this phenomenon and recession of the rosettes. The cork layer that separates the old phloem from the new appears at least a week before the peak of leaf number is reached nor does its formation interrupt the growth of new xylem and phloem in the root. The phenomenon is, however, another evidence of deep-seated developmental change during the flowering phase, and its result, the formation of an almost completely new root, suggests that it may be co-ordinated indirectly with the tendency to defoliation, which results in a more or less new crop of leaves.

It is noteworthy that annual plants in their flowering phase (Loehwing (3) ) display the same changes that have been noted for kok-saghyz, namely, reduction or cessation of growth in shoot and root, reduced water balance and increased sensitivity, especially of the older leaves, to drought. Other internal changes, as far as they have been determined, are also paralleled in the two cases. As summarized by Krotkov (1) these include hydrolysis of proteins and carbohydrates, increase in osmotic pressure, and translocation toward reproductive organs and roots. Loehwing distinguishes the flowering from the fruiting phase in annuals and reserves the term maturation for the latter, but in kok-saghyz the long overlapping of flower and fruit formation makes it difficult to separate them as two phases, so maturation has here been used to cover both.

Probably many other perennial plants of rosette habit behave in some measure like kok-saghyz. An example, closely allied it is true, is that of an unidentified species of *Taraxacum* grown side by side with the rows of kok-saghyz and measured in the same way. It displayed a similar though less severe set back after flowering.

To sum up, kok-saghyz after the peak of flowering passes into a period, which may be termed the maturation phase, in which growth is greatly reduced, if it does not cease, and sensitivity to unfavourable conditions, at least such as tend to reduce the water balance, is greatly increased. The degree of defoliation and the time that elapses before a new growth cycle begins is greater, due to inherent causes, in the second (and subsequent) years of the plant's life than in the first. It also depends on the severity of the environmental conditions referred to. Hence the more extreme setback termed 'summer dormancy' is commonly confined to plants that have passed their first year, and it requires for its development the coincidence of the maturation phase with a period of high insolation and considerable drought such as occurs only in midsummer. To this extent 'summer dormancy', though not the occurrence of maturation, is determined by environment.

As regards the suitability of the local climate for growth of kok-saghyz, to judge by the 1943 and 1944 seasons it compares favourably with that of the

zone of cultivation of the plant in Russia. Our second year plants developed rather more rapidly, completing their first flowering by the middle as compared with the end of July, and very few of them displayed the degree and duration of dormancy that is described as usual in Russia. The maturation setback, which is important even in first year plants and under our weather conditions, can best be reduced, it would seem, by selection, though cutting off the flower heads is a feasible beneficial treatment.

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## THIAMIN IN MANITOBA VEGETABLES<sup>1</sup>

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### Abstract

A survey has been made of the thiamin contents of Manitoba grown vegetables. These are of the same magnitude as of those grown elsewhere. The amounts vary with the vegetable and with the variety. There is often a wide range of values within a single variety. The effect of the district where grown is not significant. Cooking causes some destruction of the vitamin, but on an average boiled vegetables retain 63% of their thiamin, 23% is dissolved in the cooking water, and 14% is destroyed. Storage of raw vegetables does not decrease their thiamin contents appreciably. Canning causes losses, these varying with the vegetable and with the time of blanching and processing.

Vegetables—with the exception of dried legumes—are not usually classed among the better sources of thiamin or vitamin B<sub>1</sub>. However, since they comprise an appreciable part of the food we consume—more than 28% according to a recent survey (21)—the amounts they contribute to our intake of the vitamin may well be significant.

Improved methods of assay have made easier the task of finding the amounts in the various foods that make up our diet. As one would expect, the thiamin content of fresh food is not the same as that of the food when it is consumed. This is important in the evaluation of vegetables, only a part of which are consumed when newly harvested and without cooking. Storage conditions, canning, and cooking may be expected to contribute to the destruction of thiamin in them.

A large number of thiamin assays of fresh vegetables are reported in the literature. Some of the most comprehensive reports are included in Table I. Their results have been calculated to micrograms per 100 gm. The data of Booher, Hartzler, and Hewston (2) and of Fixsen and Roscoe (11) are compilations, the other data report the results of original investigations. Assays of fewer vegetables are reported by Conner and Straub (5), Farrell and Fellers (7), Fellers, Esselen, and Fitzgerald (8), Fincke (9), Fincke, Little, Redelings, and Perkins (10), Gleim, Tressler, and Fenton (12), Heller, McCay, and Lyon (16), Meiklejohn (20), Moyer and Tressler (22), and Oser, Melnick, and Oser (24). Their data are of the same order as those of Table I, with two exceptions. Fincke reports values for green peas as high as 740, while Conner and Straub report 147 as the value for squash. All of these data show that one kind of vegetable varies a great deal in thiamin content, and that there is a marked difference between different kinds of vegetables as

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TABLE I  
THIAMIN CONTENT OF FRESH VEGETABLES ( $\mu\text{GM.}/100 \text{ GM.}$ )  
(DATA REPORTED IN THE LITERATURE)

	Aughey and Daniel (1)	Booher <i>et al.</i> (2)	Fixsen and Roscoe (11)	Hamner <i>et al.</i> (15)	Lane <i>et al.</i> (18)	Nagel and Harris (23)	Pyke (26)
Artichoke	—	225-228	—	—	—	—	171-285
Asparagus	—	177-195	—	—	—	—	—
Bean, green	66	66	—	46-102	44-94	70	51- 75
Beet	—	18	—	—	25	—	—
Broccoli	—	99	—	—	—	—	72-102
Brussel sprout	—	171	—	—	—	—	—
Cabbage	—	69- 84	75-420	—	37	—	30-138
Carrot	57- 66	40- 72	180	38- 70	76	127	48-126
Cauliflower	—	90-171	330	—	—	92	45-105
Celery	—	36- 45	Trace	—	—	—	—
Chive	—	—	—	—	—	—	114
Corn	—	120-198	—	—	—	—	—
Cucumber	—	45- 54	—	—	—	—	—
Garlic	—	—	—	—	—	—	159
Horseradish	—	—	—	—	—	—	69
Kale	—	189	—	—	—	—	45- 81
Leek	—	—	—	—	—	—	96-147
Lettuce	—	69- 87	<48- 90	—	—	—	—
Onion	—	24- 33	120	—	27	—	30- 57
Parsley	—	—	—	—	—	—	171
Parsnip	—	114	—	—	—	—	—
Pea, green	450	399-450	120-300	259-354	—	392	171-327
Pepper	—	60	—	—	—	—	—
Potato	135-150	48-186	<42-180	53- 90	84	188	—
Pumpkin	—	—	30	—	—	—	—
Radish	—	39	18-180	—	—	—	39- 51
Spinach	129	105-129	60-210	67- 86	100	304	81-156
Squash	—	42- 48	30	—	—	69	—
Tomato	—	45-120	120	—	—	—	24- 69
Turnip	—	33- 60	120	—	51	128	30- 45

sources of thiamin. Of the vegetables reported, green peas appear to be the best source. Artichoke, asparagus, and corn are not quite as good. Potatoes and spinach rank below these, but are still better than many other vegetables. None of the vegetables rank as high as some of the better known sources of thiamin like lean pork with 1830 and oatmeal with 810  $\mu\text{gm.}$  per 100 gm. They are more on a par with peanut butter, eggs, chicken, and bananas whose corresponding thiamin contents are 267, 258, 111, and 54. These last six figures represent values reported by Booher *et al.* (2).

There are comparatively little data on the effect of environment on the thiamin content of vegetables. Fincke *et al.* (10) found little variation in the amounts in peas grown in different parts of Oregon during a single season, but did find a significant difference between the amounts in a single variety grown in the same locality in different seasons.

A number of workers have reported losses of thiamin from vegetables due to ordinary cooking procedures. Aughey and Daniel (1) found that as much

as 22% was destroyed and an additional 15% was dissolved in the cooking water. Cooking losses were much the same for boiled pared potatoes, spinach, green peas (cooked with soda), and snap beans. Green peas cooked without soda showed a loss of 20% (11% dissolved in cooking water) while carrots showed no change. Little or no cooking losses for fresh snap beans or for snap beans frozen for 16 months or canned for 16 months were found by Farrell and Fellers (7).

Heller, McCay, and Lyon (16) found losses of from 16 to 64% in large quantity cookery practices. These were lowered when cooking procedures were changed. Nagel and Harris (23) found that as much as 89% of thiamin was lost from food cooked and handled under restaurant conditions. The total cooking and steam table losses averaged 70%. Oser, Melnick, and Oser (24) found that losses varied with the cooking method, being from 6 to 46% in peas. Greater losses were incurred when larger amounts of water were used. They concluded that with improved methods of cooking, losses should be no greater than 10%. Lane, Johnson, and Williams (18) stress the loss of thiamin in the cooking water. Gleim, Tressler, and Fenton (12) found little actual destruction of thiamin, but solution of from 5 to 54% of it. Meiklejohn (20) concluded that steaming of potatoes extracted the greater part of thiamin from them.

There have been some investigations made of the effect of storage on thiamin content of vegetables. Langley, Richardson, and Andes (19) found no decrease in vitamin B<sub>1</sub> potency of raw carrots stored for four months in either cool or warm cellars. Gleim, Tressler, and Fenton (12) report that asparagus and spinach showed slight loss of thiamin when stored at room temperature for 24 hr., but that asparagus showed a loss of 18% and spinach 15% when stored for a week at 0 to 4.4° C.

There is little information available on the effect of commercial canning operations on the retention of thiamin in vegetables. Most studies have been made on home canning operations. Richardson and Mayfield (27) found no loss of the vitamin in carrots canned by the hot pack method in the oven, but found a slight loss when a pressure cooker replaced the oven. Fellers, Esselen, and Fitzgerald (8) found the thiamin retention of four canned vegetables to be: asparagus 72%, peas 60%, lima beans 28%, and spinach 71%. Farrell and Fellers (7) report a loss of 17% of the thiamin in green snap beans canned at the normal pH of 5.3. Fincke (9) observed a general trend toward lower thiamin contents of peas scalded for longer times and at higher temperatures. No significant difference was noted between peas blanched in steam or in hot water at the same temperature for the same length of time.

Surveys conducted by Clifcorn and Heberlein (4) indicate that blanching and processing are critical procedures in canning. Eddy, Kohman, and Carlsson (6) found that the duration and method of blanch are important. There may be further losses of thiamin in the stored canned vegetables. Farrell and Fellers (7) report losses of 45% in green snap beans after 12 months. Guerrant, Vavich, and Dutcher (14) found that high temperatures

of storage caused further destruction of thiamin in canned tomato juice, lima beans, and yellow corn.

A problem of local interest is whether Manitoba grown vegetables contain the same amounts of thiamin as those grown elsewhere, and whether these amounts are affected by the usual cooking procedures. To answer these questions a study has been made of the thiamin contents of Manitoba vegetables and the effects of processing and storage on them. An attempt has been made to study the variation in thiamin content of vegetables grown in seven districts in and close to Winnipeg.

### Experimental

Most of the vegetables used were grown in the University garden at Fort Garry. Only these were used in canning, storage, and cooking experiments. Other samples were obtained on the open market and are designated as such. To show the differences due to environment, samples of five vegetables—beans, beets, carrots, peas, and potatoes—were obtained from seven districts—Narol, Middle Church, Springfield, Winnipeg (Fort Rouge), St. James, Fort Garry, and Domain. This represents an area approximately 30 miles from north to south and 15 miles from east to west.

Vegetables from Fort Garry were analysed the same day as picked. Those from other points were collected the day before, wrapped in wax paper, and stored overnight in the refrigerator.

Analyses were made of 23 different fresh vegetables. Several varieties of some of these were tested. The results of these studies are recorded in Table II. The thiamin contents of the selected five fresh vegetables grown in seven different districts are included in Table III. The two values reported for each vegetable in Table III represent assays of duplicate samples taken from one large representative sample. The data for potatoes obtained from Springfield were completely out of line, the difference between the two determinations exceeding all other differences. Since it was not possible to get another sample from this district, owing to the lateness of the season, new values were estimated according to the method of Yates, as described by Goulden (13). These estimated values are included in Table III.

Only one cooking method—boiling—was employed. The amounts of vegetable and water used were such as to provide from two to four average sized servings. The weights of cooked vegetables and cooking water were determined. Each was assayed separately for thiamin. Results are recorded in Table IV.

The effect of storage was investigated with nine vegetables. From 40 to 60 lb. of each of beets, carrots, onions, parsnips, potatoes, and turnips, six heads of cabbage and six fruits each of pumpkin, and squash, were stored under appropriate conditions in the storage houses of the Plant Science Division. Thiamin tests were made just prior to storage. After approximately four months all vegetables appeared to be in excellent condition and

TABLE II  
THIAMIN CONTENT OF MANITOBA VEGETABLES

Vegetable	Variety	No. of samples	Thiamin, $\mu\text{gm.}/100 \text{ gm.}$		Average serving, gm.	Thiamin in average serving, $\mu\text{gm.}$
			Range	Mean		
Asparagus	Mary Washington	2	63- 64	64	50	32
	Market	2	30- 31	31	50	16
Bean	Round Pod	—	—	—	—	—
	Kidney Wax	4	49- 61	55	100	55
	Market	12	11- 75	62	100	62
Beet	Detroit Dark Red	7	9- 29	14	100	14
	Market	12	6- 31	17	100	17
Brussel sprout	Market	1	—	88	70	62
Cabbage	Danish Ball Head	4	30- 37	33	50	17
Carrot	Red Cored Chantenay	6	13- 26	20	100	20
	Market	9	24- 47	35	100	35
Cauliflower	Market	2	78- 97	88	70	62
Corn	Golden Bantam	2	109-110	110	100	110
Cucumber	Straight 8	2	22- 23	23	50	12
Kale	Market	2	49- 50	50	100	50
Lettuce	Grand Rapids	2	30- 30	30	10	3
	New York No. 12	4	17- 19	18	10	2
Onion	Yellow Globe Danvers	6	9- 29	19	100	19
Parsley	Market	2	57- 61	59	10	6
Parsnip	Hollow Crown	6	57- 83	73	100	73
Pea	Laxton Progress	2	91-102	97	100	97
	Lincoln	2	167-183	175	100	175
	Homesteader	2	183-189	186	100	186
	Market	10	176-280	211	100	211
Pepper, green	King of the North	2	36- 39	38	100	38
Pumpkin	Small Sugar	4	16- 45	31	100	31
Potato	Irish Cobbler	6	81-103	90	150	135
	Warba	6	77- 97	89	150	134
	Market	10	51- 90	68	150	102
Radish	Scarlet Globe	2	6- 7	7	10	1
Squash	Golden Hubbard	4	6- 18	13	100	13
Swiss chard	Lucullus	2	24- 26	25	100	25
Tomato	Market	2	39- 47	43	100	43
Turnip	Purple Top Swede	6	22- 68	45	100	45

Note: Vegetables labelled "Market" are unidentified varieties purchased on the open market.

TABLE III  
THIAMIN CONTENTS OF VEGETABLES FROM DIFFERENT DISTRICTS,  $\mu\text{gm.}/100 \text{ gm.}$

Districts	Vegetables									
	Bean		Beet		Carrot		Pea		Potato	
Narol	73	75	31	31	37	39	280	280	67	65
Middle Church	65	56	17	20	47	43	192	196	68	61
Springfield	62	62	23	26	24	25	176	169	60	59
Winnipeg (Fort Rouge)	35	42	7	7	39	37	173	179	79	72
St. James	64	63	6	7	24	24	189	183	83	84
Fort Garry	60	61	9	9	38	37	183	167	72	72
Domain	75	67	14	11	43	35	239	231	51	55

TABLE IV  
THIAMIN RETENTION IN COOKING VEGETABLES

Vegetable	Condition	Weight of sample, gm.	Cooking time, min.	Thiamin in sample, $\mu$ gm.		Thiamin in cooking liquid, $\mu$ gm.	Distribution of thiamin, %		
				Raw	Cooked		Retained by cooked sample	Dissolved in cooking liquid	Destroyed during cooking
Asparagus	Fresh	110	15	70	38	17	55	24	21
	Fresh	385	15	118	99	10	84	8	8
Beet	Fresh	600	20	148	32	—	22	—	—
Bean	Fresh	400	20	201	111	87	55	43	2
Brussel sprout	Fresh	300	20	263	141	56	54	21	25
Cauliflower	Fresh	300	15	263	94	43	36	16	48
Cabbage	Fresh	400	20	121	45	44	37	36	27
	Stored 4 months	340	25	84	43	37	51	44	5
Corn	Fresh	400	10	438	418	19	95	4	1
Carrot	Fresh	500	15	68	35	30	51	44	5
	Stored 6 months	500	20	125	112	46	90	37	—
	Stored 10 months	300	20	62	41	27	67	44	—
Kale	Fresh	300	30	149	108	38	73	26	1
Onion	Stored 3 months	300	15	85	65	33	77	39	—
	Stored 8 months	300	15	53	31	17	57	32	11
Parsnip	Fresh	300	10	238	149	82	63	34	3
	Stored 5 months	300	15	173	107	47	62	27	11
	Stored 8 months	300	15	245	191	48	78	19	3
Pumpkin	Fresh	500	15	97	83	15	85	15	0
	Stored 5 months	500	20	217	74	38	34	17	49
Pea	Fresh	635	20	613	315	120	51	19	30
Potato,	Fresh	300	15	297	235	78	79	26	—
Irish Cobbler	Stored 4 months	300	17	258	161	38	62	15	23
	Stored 6 months	300	17	258	226	28	88	11	1
Potato,	Fresh	300	15	233	122	32	52	13	35
Warba	Stored 3 months	300	20	273	136	30	50	11	39
	Stored 6 months	300	20	290	243	25	84	8	8
Squash	Fresh	400	10	70	50	13	71	18	11
	Stored 5 months	300	10	24	26	1	105	5	—
Swiss chard	Fresh	400	10	99	70	38	70	38	—
Turnip	Fresh	300	15	202	82	41	40	20	40
	Stored 4 months	300	15	68	61	—	89	—	—
	Stored 6 months	300	20	137	51	32	37	23	40

were analysed again. By the end of March the beets, cabbage, pumpkin, and squash showed signs of spoilage so a second analysis was not made of any of these four. The other stored vegetables were tested again. Storage conditions are detailed in Table V, and the results of the storage tests are reproduced in Table VI.

Canning studies were limited to eight vegetables—asparagus, beans, beets, carrots, corn, kale, green peas, and Swiss chard. Vegetables were canned the day they were picked, according to standard methods of canning for general household usage as outlined by Pfund (25). The jars were stored in the dark at ordinary room temperatures, and analysed from time to time. These data appear in Table VII.

TABLE V  
STORAGE OF VEGETABLES

Vegetable	Variety	Amount stored	Date stored, 1944	Storage conditions	Temperature, °F.
Beet	Detroit Dark Red, C.E.F. strain	50 lb.	Oct. 10	Cool and moist (packed in peat)	32-40
Cabbage	Danish Ball Head	6 heads	Oct. 21	Cool and moist	32-40
Carrot	Chantenay	50 lb.	Oct. 12	Cool and moist (packed in peat)	32-40
Onion	Yellow Globe Danvers, Strain No. 11	60 lb.	Sept. 25	Cool and dry	34-45
Parsnip	Hollow Crown	40 lb.	Oct.	Cool and moist	32-40
Potato	Irish Cobbler	50 lb.	Sept. 7	Cool and moist	32-40
	Warba	50 lb.	Aug. 30	Cool and moist	32-40
Pumpkin	Small Sugar	6 fruits	Aug. 28	Warm and dry	58-66
Squash	Green Hubbard	6 fruits	Aug. 28	Warm and dry	58-66
Turnip	Purple Top Swede	50 lb.	Aug. 20	Cool and moist	32-40

TABLE VI  
EFFECT OF STORAGE ON THIAMIN CONTENT OF RAW VEGETABLES

Vegetable	Variety	Fresh thiamin content, $\mu\text{gm.}/100\text{gm.}$	During storage		After storage	
			Time stored, days	Thiamin content, $\mu\text{gm.}/100\text{gm.}$	Time stored, days	Thiamin content, $\mu\text{gm.}/100\text{gm.}$
Beet	Detroit Dark Red	12	128	10	—	—
Cabbage	Danish Ball Head	31	121	35	—	—
Carrot	Red Cored Chantenay	14	201	25	292	21
Onion	Yellow Globe Danvers	—	—	28	—	18
Parsnip	Hollow Crown	79	142	58	218	82
Pumpkin	Small Sugar	19	147	44	—	—
Potato	Irish Cobbler	99	121	86	183	86
	Warba	78	107	91	176	97
Squash	Golden Hubbard	18	148	8	—	—
Turnip	Purple Top Swede	67	120	23	182	46

Thiamin was determined by the method of Hennessy and Cerecedo (17). Most duplicate analyses agreed within  $\pm 5\%$ . This is a much smaller difference than that between different lots of the same vegetable or indeed between different vegetables of the same lot. Each value reported is the average of duplicate determinations. Results are reported as micrograms of thiamin per 100 gm. of material.

TABLE VII  
RETENTION OF THIAMIN IN VEGETABLES DURING CANNING

Vegetable	Time* of blanch, min.	Time** of processing, min.	Time between canning and assay, days	Retention of thiamin, %
Asparagus	3.5	30	98 321	93 137
Beet	17	30	109 305	19 21
Bean	3	30	111 291	57 47
Carrot	3	30	104 306	109 61
Corn	2	60	125 271	13 13
Kale	3	60	111 250	40 18
Pea	3	45	104 307	29 30
Swiss chard	3	60	98 309	28 24

\* All blanching was done with water at a temperature of 212° F.

\*\* Kale and Swiss chard were processed at 250° F. All other vegetables were processed at 240° F.

### Discussion

The results of the analyses of raw vegetables are presented in Table II. The weights for average servings are those of Bowes and Church (3). The amount of thiamin in an average serving is calculated on a raw basis. This is likely more than there would be in an average serving of the cooked or canned vegetable.

The thiamin contents of the vegetables analysed are of the same order as those reported by other investigators. They range from a low of 6 gm. for radish to a high of 280 gm. per 100 gm. for peas. Of those vegetables examined, peas are the best source of thiamin, followed by corn. Potatoes, which are consumed in rather large quantities, are better than most other vegetables studied. Brussels sprouts, cauliflower, and parsnips have somewhat less thiamin than the vegetables mentioned. In some cases—particularly for peas—there is a marked difference between varieties of the same vegetable. There is often a considerable range for a single variety. This is obvious in those cases where a larger number of samples were available for analysis. Other vegetables might be expected to show a like variation had sufficient of them been assayed. In all varieties of beets, onions, and pumpkin studied, the highest value is almost three times that of the low. The amounts

of thiamin in average servings vary as do the amounts of thiamin in the fresh vegetables. The contribution of potatoes is emphasized because of the amounts of them consumed. But certainly those vegetables that are better sources of thiamin are contributing appreciably to our daily intake.

The data of Table III show the thiamin contents of five selected vegetables from seven districts. The actual amounts are similar to those reported in Table II. There is a difference between districts for any one vegetable. However, no one district is uniformly high or low. This point was studied further by making an analysis of variance of these data. The  $F$  value for districts was 1.57, which is well below the 5% point of 2.51. This indicates that the thiamin content of the vegetables does not vary significantly over the district studied. There is the possibility that this finding would be different if a larger or more varied district were subjected to a similar survey. Still, our finding is in harmony with that of Fincke *et al.* (10) who worked with a larger area.

The results of the cooking tests appear in Table IV. In each test analyses were made of the raw sample, the cooked sample, and the cooking liquid. The thiamin of the raw sample that is not accounted for in the cooked sample and in the cooking liquid is labelled "destroyed during cooking". Each cooking test is reported separately.

The results show marked differences in the amounts of thiamin destroyed. Several vegetables retain all of their thiamin, either in the vegetable or in the cooking liquid. Some, like cauliflower and turnip, show as much as 40% destruction. Stored pumpkin shows greater destruction than fresh. These increased amounts destroyed do not seem related to cooking times. The results as a whole do not warrant drawing definite conclusions. On the average vegetables retained 63% of their thiamin, and retained as well another 23% in the cooking liquid. The remaining 14% was destroyed. This does emphasize the importance of saving all cooking liquids and using them in such products as gravies, sauces, and soups.

The results of storage tests are recorded in Table VI. This shows thiamin in the fresh vegetables and after varying times of storage. There is no orderly change in the amounts of thiamin. However there does not appear to be any marked decrease in the amounts of the vitamin in the vegetable at the end of six to seven months' storage. Apparent increases are probably due to variability in the vitamin contents of the vegetables themselves.

The data of canning tests are recorded in Table VII. No assays were made on the vegetables immediately after canning. The first ones made on the canned vegetables show in some cases a marked drop in thiamin content. In only two cases do the later retention tests differ appreciably from the first. In each case one of the values is greater than 100%, which must be due to variability in the material itself. It would seem likely that much of the initial drop must have been due to the canning itself.



Beets and corn showed the most severe canning losses. Peas, Swiss chard, and kale exhibited low thiamin retention. Beans showed a retention of 57%, while asparagus and carrots retained most of their original thiamin. Losses were greatest for those vegetables having longer blanching or processing times. On further storage carrots lost some of their thiamin, kale and beets showed a smaller loss, while the other vegetables appeared to retain the balance of the vitamin left after canning.

### Acknowledgments

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# A SEEDLING BLIGHT AND LEAF SPOT OF FLAX CAUSED BY *CONIOTHYRIUM OLIVACEUM* BON.<sup>1</sup>

BY T. C. VANTERPOOL<sup>2</sup>

## Abstract

*Coniothyrium olivaceum* Bon. was found to be the cause of a seedling blight of flax in 1945 and 1946 in Saskatchewan. Twelve out of 32 seed samples of flax from the 1945 crop selected for their low germination were carrying an average of 8% seed-borne infections. Leaf-spot lesions were found during late August, 1946. The wide distribution of the fungus suggests that it has been present in the province for some time. Hitherto, it has been reported on flax from the Argentine only. A strain of the same organism was present as a trace in one sample of sweet clover seed. Pycnidia develop abundantly in culture. The spores measure  $7.5-10$  ( $11.5$ )  $\mu \times 4.2-7.5 \mu$ , averaging  $9.1 \times 6.0 \mu$ , which is somewhat larger than the average for the species, but yet within the range limits. The disease is favoured by high moisture and probably by cool temperatures. It is one of the minor diseases on flax.

In June, 1945, *Coniothyrium olivaceum* Bon. was found to be one of the chief fungi associated with a seedling blight of linseed flax growing on summer-fallowed land following crested wheat grass (*Agropyron cristatum* (L.) Gaertn.), at the Dominion Experimental Station, Scott, Sask. *Rhizoctonia Solani* Kühn was present in about equal amounts, and *Fusarium Scirpi* Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr. less commonly. *C. olivaceum* proved to be moderately pathogenic to flax seedlings in artificial inoculations in the laboratory and in the greenhouse. During the following winter it was isolated from 12 samples of seed from the 1945 flax crop from widely scattered districts of the province. In the spring of 1946, it was isolated from blighted seedlings from three separate localities, and during the latter part of August it was found causing a leaf spot (Pl. I, Figs. A and B) on Crystal flax in a localized area in the University plots. These spots were brought to my attention by E. J. Hawn, student assistant.

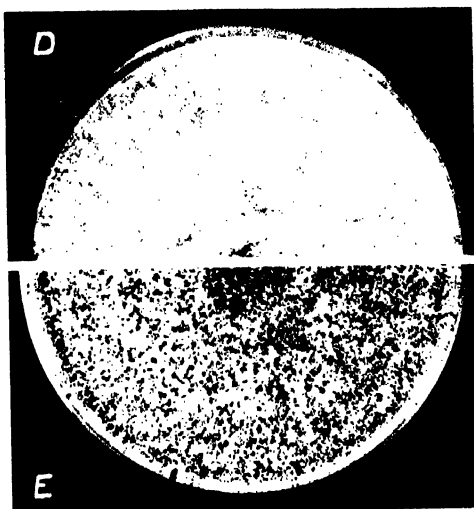
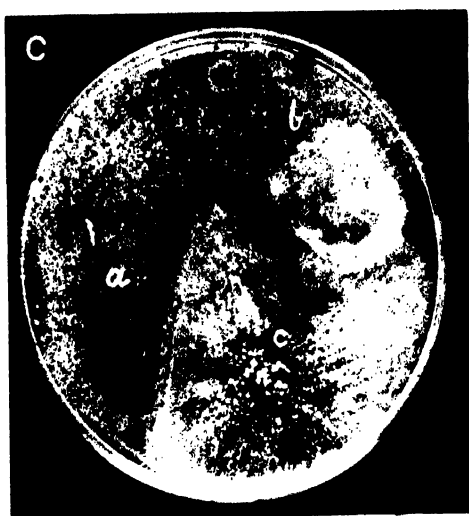
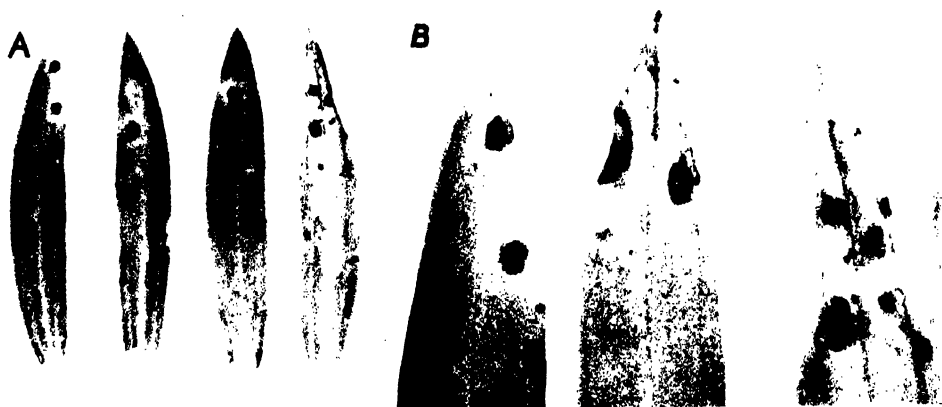
The foregoing observations suggest that the organism has been present in Saskatchewan for some time, possibly on another host. *C. olivaceum* was reported by Wollenweber and Hochapfel (6) in 1937, on the stems of flax from the Argentine. The damage it causes on flax is at present of minor importance,

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Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask., with financial assistance from the Saskatchewan Agricultural Research Foundation.

<sup>2</sup> Professor of Plant Pathology.

FIG. A. Leaf spots caused by *Coniothyrium olivaceum* on Crystal flax from field collections; natural size. FIG. B. Leaf spots enlarged  $3\frac{1}{2}$  times. FIG. C. Portions of disinfected, blighted flax seedlings on malt-extract agar showing at a, *C. olivaceum*; b, *C. olivaceum* and *Rhizoctonia Solani*; and c, *R. Solani*. FIG. D. A seven-day culture on malt extract agar. FIG. E. The same after 32 days showing numerous pycnidia. FIG. F. Pycnidia on a six weeks' culture on malt-extract agar;  $\times 10$ . FIGS. G and H. Old isolation plates showing mature pycnidia on portions of flax seedlings;  $\times 10$ .





but its behaviour during 1945 and 1946 in Saskatchewan warrants the publication of this paper.

### Isolations

The pathogen was obtained in culture from blighted flax seedlings from Scott in 1945, and from Saskatoon and Hudson Bay Junction in 1946; from lesioned cotyledons from Nipawin in 1946; from 12 out of 32 flaxseed samples selected for their low germination at the Seed Laboratory, Plant Products Division, Saskatoon; from leaf lesions on Crystal flax in August, 1946; and from one sample of sweet clover (*Melilotus alba* Desr.) seed where it was present as a trace only. The calcium hypochlorite method of disinfection as described by Wilson (5) was used, the seed being soaked, with intermittent shaking, in the stock solution for 10 min., and then plated out. Immersion for one to two minutes in the alcohol-mercuric chloride mixture of Machacek and Greaney (2), followed by three rinses in sterile water, also proved satisfactory. The fungus was grown in flasks of crushed oat hulls (sold as 'Vim' stock feed) for large-scale greenhouse and field tests. Spore suspensions were readily secured from cultures on the ordinary laboratory media, where it fruited profusely (Pl. I, Fig. E).

### The Disease

*C. olivaceum*, besides being moderately pathogenic on flax, also caused moderate seedling blight on *Linum grandiflorum* Desf. in artificial inoculations. Both the sweet clover and flax strains were weakly parasitic on sweet clover in artificial inoculations. No definite parasitic ability was shown towards alfalfa (*Medicago sativa* L.), brome grass (*Bromus inermis* Leyss.), crested wheat grass, Argentine rape (*Brassica Napus* L.), sunflowers (*Helianthus annuus* L.), or wheat (*Triticum aestivum* L.).

The disease on flax seedlings is of the same damping-off and blighting type commonly caused by *Rhizoctonia Solani* (3 and 4, p. 21), with *Pythium* and *Fusarium* spp. sometimes also associated. Affected seedlings usually dry up and are commonly buried by soil during subsequent spring rains. It is probable, therefore, since the parasite is internally seed-borne and may be isolated from diseased cotyledons (*vide supra*) that it develops pycnidia on the cotyledons of seedlings from which spores are disseminated for later leaf infections (Pl. I, Figs. A and B); these, in turn, supply inoculum for boll and seed infections. No pycnidia have been observed on seed from naturally infested samples, but they develop readily on infected seeds plated on nutrient media.

The leaf lesions appear to be confined to the top third of the leaf, being more common near or at the plane or straight margin of the leaf than near the convex margin (Pl. I, Figs. A and B). The spots are subspherical to oval with fairly regular margins, brown to dark brown in colour, and slightly roughened, especially in older lesions where pycnidia have formed. Adjoining areas soon turn yellow. Affected leaves fall off prematurely.

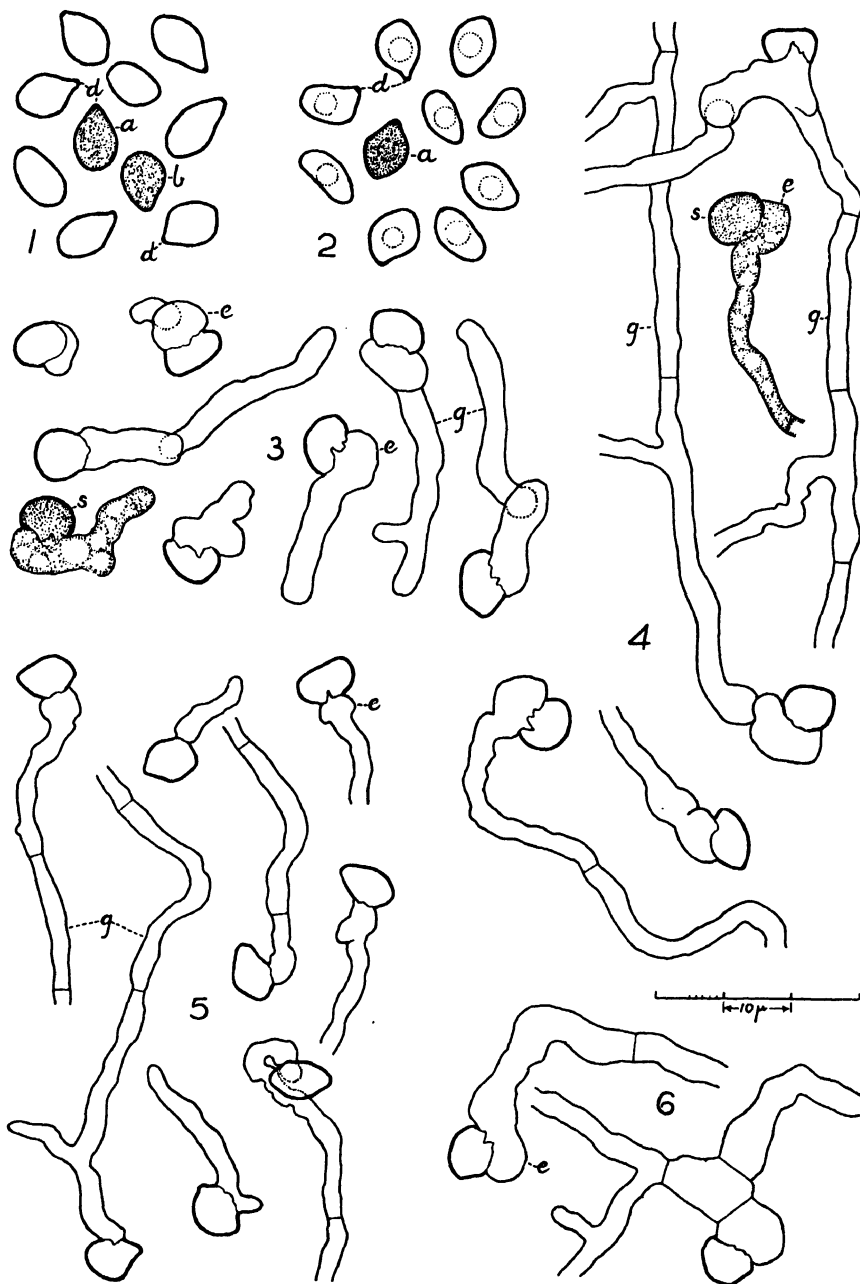
The damage caused to flax is of minor importance at the present time. If weather conditions favour heavy seed infection in the bolls, a slight reduction

in seedling stand might be expected when such seed is sown. The diseased areas on leaves were relatively smaller than diseased areas caused by pasmo (*Sphaerella linorum* Wr.) or browning (*Polyspora Lini* Laff.). Defoliation appeared to be hastened, but the damage caused is considered negligible especially in the light of Klages's experiments (1) on the effect of defoliation at various stages of growth on the yield of flax.

### The Pathogen

The pathogen develops readily on cornmeal, malt extract, and potato-dextrose agars when naturally infested seed and seedlings, suitably surface sterilized, are plated in the usual manner (Pl. I, Fig. C, *a* and *b*). The growth is at first a dull, creamy white, with a mat surface; later, the mycelium becomes slightly aerial and orange-coloured in patches. Slight variations in mycelial densities give a radiating aspect to the growth on an agar plate (Pl. I, Fig. D). After about five days pycnidia begin to form, scattered indiscriminately over the surface of the agar (Pl. I, Fig. E). These are black, subspherical to irregular, and single or coalesced in groups (Pl. I, Fig. F) from which two or three ostioles may arise from a single chamber. The ostioles are apical or slightly to one side. Under high moisture conditions short, black columns of conidia may ooze through the ostioles, finally enveloping the pycnidia in a shining, viscid, black mass. Pl. I, Figs. G and H show pycnidia that have developed on portions of naturally infested flax seedlings after disinfecting and plating in the customary manner; in Fig. H, the seedling is more submerged in the agar than in Fig. G.

The majority of pycnidia fall within a diameter range of 250 to 350 $\mu$ , with extremes of 180 and 600 $\mu$ . The conidia are one-celled, thick-walled, brown to olivaceous, angularly ovate to napiform in surface view and flattened elliptical in side view (Figs. 1 and 2), measuring 7.5–10 (11.5) $\mu$   $\times$  4.2–7.5 $\mu$ , with an average of 9.1  $\times$  6.0 $\mu$ . In water mounts the contents are finely granular or slightly globular; in lactophenol the contents appear pale olivaceous brown, homogeneous or very slightly granular with a darker brown, spherical central globule. Certain light conditions frequently produce a dark-area effect or spot in the cell wall at the narrow, sometimes truncate, end (Figs. 1 and 2, *d*). Wollenweber and Hochapfel (6) record the occasional presence of a single septum in spores prior to germination, but no septa have been observed in the Saskatchewan form in germination studies. Germination occurs readily. The germ tube ruptures the cell wall and soon forms a characteristic swelling adjacent to the spore (Figs. 3, 4, and 5, *e*). Both the swelling and the hyphae are more robust on nutrient media (Figs. 3, 4, and 6) than in water or on plain agar (Fig. 5). In 24 hr., mycelial development is from three to six times as copious as that shown in Figs. 4 and 5. Radial growth rate is 11 mm. on cornmeal agar, 12.5 mm. on malt-extract agar, and 15 mm. on potato-dextrose agar in 24 hr. at 22° C.



FIGS. 1 to 6. *Coniothyrium olivaceum*. FIG. 1. A water mount of spores from malt-extract agar; a, contents homogeneous or finely granular; b, contents slightly globular; d, dark spot in cell wall under certain light conditions. FIG. 2. A lactophenol mount showing a single globule in each spore; a, contents homogeneous. FIG. 3. Germination on cornmeal agar after 12 hr.; s, spore; e, enlargement, and g, germ tube. FIG. 4. Germination on cornmeal agar after 24 hr. FIG. 5. Germination on plain agar after 24 hr. FIG. 6. Germination on malt extract agar after 24 hr. Magn.  $\times 800$ . Drawn with the aid of a camera lucida.



## Pathogenicity

Pale reddish-brown, necrotic lesions are formed on the roots of seedlings on moist filter paper in Petri dishes when discs from culture plates are used as inoculum. Complete inhibition of germination is rare. Flax grown in the greenhouse during the winter and spring months in sterilized soil in pots inoculated with oat-hull inoculum showed slight stunting, with one to three seedlings usually, in a pot of 10, becoming sickly and finally blighting completely. *C. olivaceum* was re-isolated from such seedlings. In a similar test conducted during the summer, no outstanding difference could be observed between inoculated and uninoculated plants. No disease developed in 1946 field plantings made from naturally infested 1945 seed or from inoculations of Redwing, Royal, Victory, and Viking flax with oat-hull inoculum scattered in the seed rows. The soil was dry at the time of sowing, with moisture conditions below normal until about mid-summer. The leaf infections found on Crystal followed a wet period in August; however, the infestation remained light and localized owing probably to subsequent dry atmospheric conditions.

In general, information on ecological conditions favouring the disease is meagre. Of the 12 infected seed samples from the 1945 crop, nine came from the park zone and three from the open prairie, possibly indicating that higher moisture favours the disease. There was an average of 8% seed-borne infection with a range from 1 to 32%. It is probable that the organism also overwinters in the pycnidial stage in infested leaves. According to Wollenweber and Hochapfel (6) pycnidia are formed on the stems of flax in the Argentine, but none could be found at Saskatoon on the stems of the Crystal flax having leaf lesions in late August. Dry atmospheric conditions doubtless prevented their development.

The observations recorded in this paper suggest that seed disinfection and crop rotation should serve as practical control measures.

## Acknowledgment

My thanks are due to Dr. G. R. Bisby, Imperial Mycological Institute, Kew, England, for identifying the fungus and for drawing my attention to Reference 6.

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## TETRAPLOID *TARAXACUM KOK-SAGHYZ*

### III. ACHENE WEIGHT, FLOWERING, AND PLANT DEVELOPMENT<sup>1</sup>

BY M. W. BANNAN<sup>2</sup>

#### Abstract

The ultimate size of diploid or tetraploid kok-saghyz plants, when grown in pots or at wide spacings in the field, was not determined primarily by the weight of the achenes from which they originated. Conversely large or small tetraploids when crossed among themselves did not produce achenes reflecting the differences in plant size. Tetraploids differed from diploids in possessing larger organs, but increase in size was more or less counteracted by reduction in numbers. This was especially true as regards flowering. Tetraploids were notably deficient in the production of inflorescences during the first year, and since they were correspondingly freed from the inhibiting effects of flower and seed development on root enlargement, the root size of the vegetating tetraploids tended to surpass that of the more floriferous diploids, unless the latter were disbudded. On the whole first-year plants with the broadest and leafiest rosettes and few or no capitula developed the biggest roots. An association of such characteristics is probably the best criterion for the selection of large-rooted plants. When large root size is desired it would seem advisable to avoid pregermination treatments that hasten maturation and stimulate flowering.

#### Introduction

In previous articles (1 and 2) the production of tetraploid kok-saghyz by the use of colchicine, and the characteristics of parental and  $F_1$  tetraploids were described. The present paper deals with investigations on the weight of achenes and size of plants, and also with the correlation of the various morphological characters from the standpoint of their usefulness in the selection of desirable plants and their bearing on the agronomic possibilities of tetraploids.

#### Methods

To supplement the data on  $F_1$  plants acquired during 1945, further plantings, both of the same and additional crosses, were made in 1946 from seed then two years old. The achenes, which had been stored at room temperature, were not chilled prior to germination. In March 1946 they were weighed, placed on moist filter paper in Petri dishes, and when the radicles protruded, the seedlings were set out singly in sterile soil in  $2\frac{1}{2}$  in. pots. Because inclement

<sup>1</sup> Manuscript received December 10, 1946.

Contribution from the Department of Botany, University of Toronto, Toronto, Ont., with financial assistance from the National Research Council of Canada and the University of Toronto.

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weather delayed the preparation and decontamination of the garden plot, the plants remained in the small pots until mid-June. As a consequence the seedlings were forced into a premature maturation, and many began to flower. When finally transplanted in the plot, at foot spacings in rows 18 in. apart, vegetative growth was resumed and enlargement of the rosettes proceeded rapidly. After the attainment of maximum size in late August and early September, many of the plants flowered again, but head production was not heavy. The plants were harvested in mid-October. Although the roots were dug with care, there was unavoidable breakage of the distal portions of the root systems. By mutilation of the roots of potted plants to make them comparable with roots removed from the plot, it was estimated that 15 to 20% of the roots of field plants was left in the ground. That loss in weight should be taken into account if field and potted plants are compared. After the plants were removed from the ground the leaves were twisted off, but the crowns were left intact so that any plant selected could be repotted for possible future use. The root weights presented in this paper thus include the roots and crowns, as illustrated in Fig. 6.

Parallel series of diploids and tetraploids, grown from selected large and small achenes, were also established. The diploid seed, harvested in 1945, was obtained from Dr. L. Truscott of the Ontario Agricultural College, Guelph, Ont. The tetraploid seed was a general collection from open-pollinated plants of various  $F_1$  populations also grown in 1945. The seed was stored over winter at room temperature, and was not given any pregermination chilling. From both  $2n$  and  $4n$  seed collections, the longest and shortest plump achenes were selected. Only well-filled achenes were chosen, all thin or flat specimens being discarded regardless of length. Four lots of large and small, diploid and tetraploid achenes were germinated in mid-March (Series A), and another four lots (Series B) were started two weeks later. The seedlings were set out singly in  $2\frac{1}{2}$  in. pots and later transferred to 8 in. pots. The plants of Series A were moved outside in mid-June, and the pots sunk in the greenhouse yard, while those of Series B were kept in the greenhouse throughout the summer. The plants outside were given water as required, those in the greenhouse were watered every day. The greenhouse plants, exposed to very high temperatures during July and August, grew little during the later part of the summer and produced much smaller roots than the plants outside. The roots of all plants were harvested in early October.

### Weight of Achenes and Plant Development

Data on weight of achenes and size of the resulting plants are presented in Tables I and II for the  $F_1$  progenies of specific crosses grown in 1945 and 1946, and in Table III for  $F_2$  plants derived from large or small achenes selected from a general seed collection. In the case of the 1946 plantings in the garden plot (Table I) there appeared to be a tendency for the  $F_1$  progenies derived from large achenes to have the biggest roots, as illustrated by the cross 578 X

TABLE I

TETRAPLOID  $F_1$  PROGENIES GROWN IN THE GARDEN PLOT (1946)

Cross	No. of plants	Av. weight of achenes, mgm.	Av. no. of green leaves at maximum	Av. diam. of rosette at max., in.	Av. fresh weight of roots, gm.
578 × 583	27	0.93	52.0	12.7	41.5
629 × 578	18	0.92	49.0	12.5	26.1
578 × 603	19	0.89	45.6	13.7	31.6
31 × 145	15	0.86	47.3	11.3	32.6
71 × 107	30	0.80	32.4	10.0	30.0
31 × 314	18	0.79	46.0	11.6	22.8
257 × 107	33	0.78	46.3	12.0	33.4
17 × 257	6	0.77	25.5	7.4	13.8
578 × 629	27	0.74	72.6	13.8	35.1
107 × 17	15	0.74	33.6	9.2	27.6
621 × 436	18	0.71	30.5	9.7	15.9
612 × 631	14	0.69	31.5	10.1	19.0
107 × 71	7	0.68	32.2	9.9	39.0
31 × 21 (A)	9	0.68	25.0	7.3	17.0
625 × 578	11	0.68	35.4	9.7	16.0
625 × 634	11	0.66	35.2	12.0	16.5
612 × 578	17	0.64	66.1	12.9	23.3
31 × 21 (B)	19	0.62	28.9	7.4	17.1
31 × 223	12	0.54	50.0	12.2	25.2
31 × 195	18	0.49	36.5	10.9	17.4
217 × 270	15	0.48	44.2	15.0	29.7
31 × 17	15	0.45	28.9	9.3	16.3

TABLE II

TETRAPLOID  $F_1$  PROGENIES GROWN IN 8-IN. POTS (1945 AND 1946)

Cross	No. of plants	Av. weight of achenes, mgm.	Av. no. of heads	Av. no. of leaves at max.	Av. diam. of rosette at max., in.	Av. fresh weight of roots, gm.
578 × 562 (1945)	27	0.98	12.8	33.5	9.7	20.1
629 × 619 (1945)	4	0.86	18.5	31.8	10.5	11.9
21 × 107 (1945)	17	0.76	4.5	23.3	7.9	17.9
21 × 296 (1945)	29	0.75	17.1	27.3	9.2	15.5
296 × 21 (1945)	8	—	11.9	28.3	9.0	10.0
71 × 86 (1946)	17	0.75	0	31.9	12.7	29.5
71 × 86 (1945)	16	0.74	14.4	26.6	8.8	20.1
21 × 31 (1945)	19	0.71	19.3	27.8	9.3	12.6
86 × 47 (1946)	22	0.70	0.3	30.6	11.7	18.7
86 × 54 (1946)	8	0.63	0	40.0	16.0	35.0
31 × 21 (1945)	26	0.61	23.8	30.8	9.6	16.7
619 × 629 (1945)	16	0.58	12.5	26.7	8.8	16.7
31 × 17 (1945)	33	0.57	7.4	24.4	8.0	21.0
20 × 265 (1945)	15	0.57	13.0	23.3	8.0	13.6
71 × 17 (1945)	29	0.55	8.4	23.8	8.4	18.3
86 × 71 (1946)	17	0.53	0.2	31.5	13.4	30.5
86 × 71 (1945)	28	0.52	10.2	32.1	9.4	23.5
86 × 70 (1946)	41	0.52	0.4	32.3	11.9	21.5
70 × 86 (1945)	14	—	12.7	31.2	9.6	23.2
31 × 257 (1945)	17	0.51	11.8	34.9	10.1	24.1
86 × 31 (1946)	40	0.44	0.2	28.7	11.2	23.5
31 × 86 (1945)	38	—	9.1	38.8	9.9	24.3

TABLE III

COMPARISON OF DIPLOIDS AND TETRAPLOIDS GROWN FROM LARGE AND SMALL ACHENES

Series	Type	No. of plants	Av. weight of achenes, mgm.	Av. no. of heads	Av. no. green leaves at max.	Av. diam. rosette at max., in.	Av. fresh root weight, gm.
<i>A</i> (moved outside in June)	<i>2n</i>	26	0.54	31.3	98.3	11.9	26.6
		32	0.33	42.9	97.3	12.6	27.1
	<i>4n</i>	39	0.91	4.2	38.7	11.9	34.8
		40	0.57	3.5	35.3	11.5	27.8
<i>B</i> (kept in greenhouse all summer)	<i>2n</i>	39	0.50	17.3	63.0	12.0	9.4
		42	0.34	32.6	60.5	11.1	10.7
	<i>4n</i>	39	0.90	2.8	34.1	11.4	17.5
		50	0.62	1.7	32.4	12.0	15.2

583. However, an examination of the data reveals many outstanding exceptions, as for instance the  $F_1$  of  $107 \times 71$  where the achenes were of moderate size and the roots very large. In Table II, which records the results of the plantings of different  $F_1$  progenies in pots, no parallel between achene weight and plant size is apparent. In fact, the trend here was in the opposite direction, some of the largest plants developing from small achenes. Finally, in the parallel Series *A* and *B* grown from selected large or small achenes, no constant differences in ultimate size of rosettes or of roots were discovered (Table III). Actually the diploids grown from small seed had slightly larger roots, whereas the reverse was true in the tetraploids.

The data in Tables I and II indicate that such morphological characters as number of leaves, diameter of the rosette, amount of flowering, and size of the roots are to some extent heritable, though they are also, of course, profoundly modified by the environment. For instance, the  $F_1$  progenies of  $31 \times 21$ , whether planted in pots or in the garden plot, produced roots below average size, the average weights in three plantings being 17.0, 17.1, and 16.7 gm. In contrast the  $F_1$  progenies of plants numbered 86, 107, and 587 developed roots much exceeding average size. The wide differences in number of leaves at the maximum development of the rosette in July and August, when the averages of the different families ranged from 25.5 to 72.6, and the similar differences in the diameter of the rosette are probably also to be interpreted as indicative of the operation of an inheritance mechanism. Similarly the weight of the achenes centered about different values for the various seed parents, although varying with the pollen parent. In some instances large achene size was associated with vigorous plant growth. This was true in the progeny of Plant 578, and to a lesser extent in the progeny of 107. On the other hand the achenes in most of the crosses involving Plant 86 were small, but the  $F_1$  progeny were large. Evidently there may be various assortments of the size factors for the different morphological characters, and large size of achenes is not necessarily associated with bigness of plant.

Records were kept of the rosette characteristics of plants in Series *A* and *B* to determine whether achene weight had an influence on rate of growth. In the diploids the rosettes of seedlings derived from large achenes were slightly bigger than those of seedlings grown from small seed, the average differences in numbers of leaves and diameter of the rosette at 20 to 30 days being 10 to 20%. As the plants matured these differences disappeared. However, in close plantings it might be expected that the derivatives of large achenes would be at an advantage because of their more rapid early growth. In the tetraploids no constant differences in the rosettes were observed between the derivatives of large or small achenes during early growth, though at maturity the plants grown from large achenes had slightly bigger roots.

### Size of Plants and Weight of Achenes Produced

After the roots of the  $F_1$  tetraploids were weighed in the autumn of 1945 the largest and smallest were repotted, and when flowering ensued in the spring of 1946 the large and small plants were crossed among themselves. The average weight of the achenes produced in 80 cross-pollinations between large plants, whose roots weighed 36 to 79 gm. at the end of the first season's growth, was 0.65 mgm. The weight of achenes resulting from crossings between small plants, with roots of 8.5 to 10 gm., was the same, namely, 0.65 mgm. The achene weight fluctuated widely in different plants, the average weights in various crosses tending to cluster about different values specific for each seed parent, but these differences were not related to plant stature. Similar fluctuations were noted in average achene weight in both large and small plants.

### Correlation of Morphological Characteristics

In general, plants with the widest and leafiest rosettes produced the biggest roots (Table IV), the trends in the 1946 plantings repeating those

TABLE IV

RELATIONSHIPS BETWEEN FRESH ROOT WEIGHT, HEAD PRODUCTION, NUMBER OF LEAVES, AND SIZE OF THE ROSETTE IN FIRST-YEAR DIPLOIDS AND TETRAPLOIDS

Type	Root weight, gm.	No. of plants	Av. no. of heads	Av. no. of leaves at max.	Av. diam. of rosette at max., in.
<i>2n</i>	To 19.9	13	52.2	91.7	11.2
	20-29.9	25	38.2	93.7	11.8
	30-39.9	16	29.3	113.3	13.3
	40-49.9	4	7.0	106.8	13.8
<i>4n</i>	To 19.9	16	6.3	31.8	9.6
	20-29.9	25	6.4	37.1	11.8
	30-39.9	16	2.4	36.8	12.2
	40-49.9	16	0	50.5	13.4
	Over 50	6	0	48.5	12.8



observed in 1945 and reported in a previous paper (2). However, individuals were highly variable and some deviated widely from the general trend (Figs. 1 and 2). For instance, among the diploids illustrated in Fig. 1, the plant with

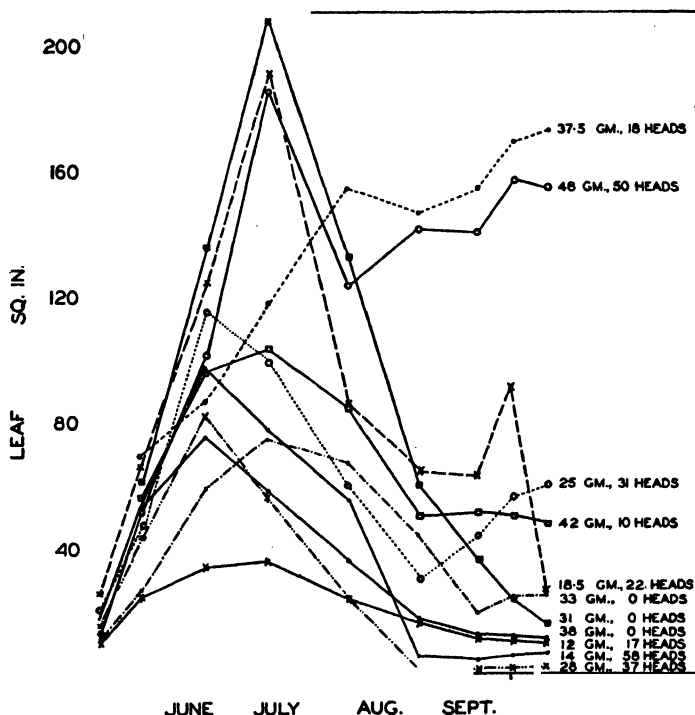


FIG. 1. Seasonal alterations in leaf area in representative first-year diploids grown from large achenes, Series A. Root weight and head production of each plant are indicated to the right.

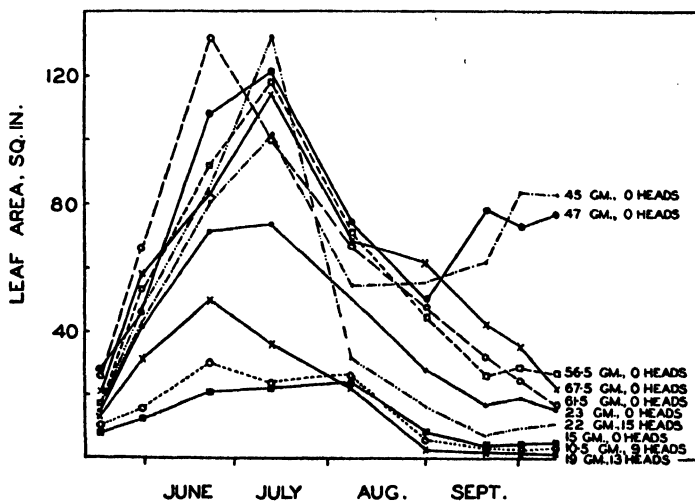


FIG. 2. Seasonal alterations in leaf area in representative first-year F<sub>2</sub> tetraploids grown from large achenes, Series A.

the greatest leaf area (208 sq. in.) developed a root weighing 31 gm., whereas other individuals with less than half that leaf area (75 and 77 sq. in.) produced slightly larger roots weighing 33 and 38 gm. It might be noted that all three plants were alike in their lack of flowering.

The seasonal alterations in number and width of leaves are illustrated in Fig. 3 for first-year diploids and tetraploids of Series A. The number of

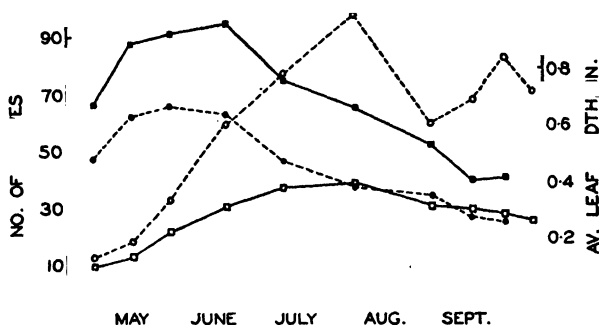


FIG. 3. Seasonal trends in number of leaves and leaf width in first-year diploids and  $F_2$  tetraploids, grown from large achenes, Series A. The leaf width indicated is the average width of all leaves, growing and mature, in a sector of the rosette, measurements being made across the broad distal portion of each leaf with correction for amount of lobing.

- number of leaves, 4n;
- number of leaves, 2n;
- leaf width, 4n;
- leaf width, 2n.

leaves increased rapidly to a maximum at 90 to 140 days, declined sharply as senescence overtook new growth and in the diploids increased again in the autumn. The amount of rejuvenescence varied greatly in different individuals, some undergoing little or no revival while others produced more leaves than during the early summer. However, owing to the small size of the autumnal leaves, leaf area generally remained at a low level during rejuvenescence (Fig. 4). On the whole, plants that attained their maximum leaf area earliest in the spring tended to flower first and to have small roots, but many variants occurred. No relationship was detected between the degree of autumnal revival and root size. In some of the large-rooted specimens a marked increase in leaf area ensued during rejuvenescence, while in others there was a steady decline (Figs. 1 and 2). Under some circumstances the decline in leaf area during the summer was more gradual in tetraploids than in diploids. This was true in Series B, where the plants remained in the greenhouse during the summer. The tetraploids were less adversely affected by the high temperatures and insect infestations, leaf area diminished less rapidly and the roots were substantially larger than in the diploids.

Leaf form is highly variable in kok-saghyz and attempts have been made to determine the causal factors in this variation and to ascertain whether there is relationship to root weight. That the environment greatly influences leaf shape has been suggested or demonstrated by various authors. For

instance, Goebel (4) has remarked that the leaves of well nourished *Taraxacum* plants are more deeply cut than in depauperate types. In certain of the Russian reports, reviewed by Krotkov (5), the statement is made that favourable conditions cause an increase in lobing in kok-saghyz. Sears (10) has

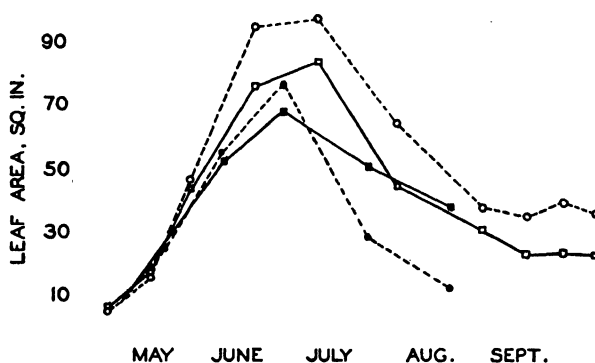


FIG. 4. Seasonal trends in the average leaf area of all first-year diploids and tetraploids grown from large achenes, Series A (plants moved outside) and Series B (plants kept in greenhouse).

□—□ Series A, 4n;  
 ○- -○ Series A, 2n;  
 ■—■ Series B, 4n;  
 ●- -● Series B, 2n.

described the ontogenetic sequence in leaf shape in *Taraxacum*, pointing out that dissection usually increases with maturation, whereas rejuvenescence restores the juvenile unsegmented type. Most kok-saghyz plants exhibit increased lobing during the rapid expansion of the rosette, but in occasional plants the leaf margins remain entire or become only slightly sinuate. Evidence presented in a previous article (2) indicates that these differences between mature plants are due in part to heredity, the leaves of  $F_1$  plants replicating the parental types. Evidently leaf shape is determined not by any single influence but rather by the interplay of ontogenetical, environmental, and genetical factors.

Some contradictory reports have appeared in the literature (Krotkov (5)) on the relationship between leaf form and root size. Some authors have observed that plants with large roots tended to have deeply cut leaves, and suggested selecting plants with lobed rather than simple leaves. Yet other authors have discounted leaf form as a useful prognostic character for selecting large-rooted plants. An examination of a few hundred  $F_1$  tetraploids grown in pots in 1945 (2) failed to reveal a correlation between leaf shape and root size. However, in the 1946  $F_2$  plantings from random seed, both diploids and tetraploids exhibited a definite trend (Table V), plants with dissected leaves of Types IV and V (Fig. 5) more often developing large roots than specimens with simple leaves. Further, in the more vigorous Series A a slightly larger proportion of the plants had deeply pinnatifid leaves than in the retarded Series B. An analysis of the 1945 data also revealed a slightly higher incidence

TABLE V

FRESH ROOT WEIGHT AND LEAF TYPE IN FIRST-YEAR, POTTED PLANTS

Series	Type	No. of plants	Av. root weight in grams of plants with leaf types as indicated				
			I	II	III	IV	V
A (moved outside)	2n	55	19.5	25.0	24.9	26.4	30.7
	4n	77	15.0	20.3	30.6	35.0	44.7
B (kept in greenhouse)	2n	73	—	8.3	8.8	10.7	11.6
	4n	89	—	11.8	15.5	18.3	20.5

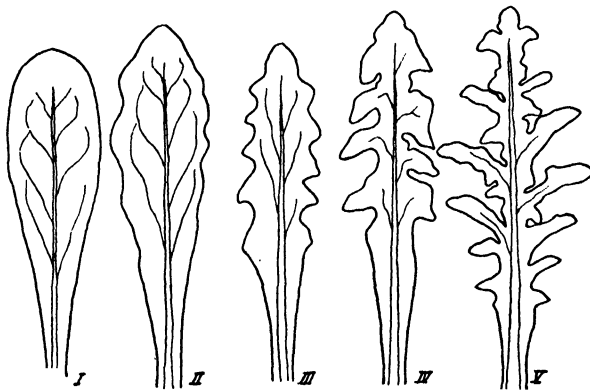


FIG. 5. Types of leaves in mature tetraploids.

of deeply lobed leaves among the more vigorous field plants than in potted specimens. These observations, on the whole, give some support to the theory that a favourable environment and luxuriant vegetative growth influence leaf form and increase lobing. It should be emphasized, however, that the average differences in leaf shape between the contrasted groups were often so slight, and individual plants deviated so widely from the general trend that leaf form must be considered of no practical value for detecting large-rooted specimens in a general population.

The relationship between flowering and vegetative characters has also been much studied. Mashtakov *et al.* (6) reported that among crowded first-year diploid kok-saghyz, flowering plants produced larger roots and tops than non-flowering plants. On the other hand, other Russian authors (see Krotkov (5)) believed absence of flowering during the first year to be associated with large root size. Recently, Scarth *et al.* (9) have observed that disbudded 2n plants surpassed those that were allowed to produce seed. Roberts and Struckmeyer (8), working with a number of genera, including *Cineraria*, found flowering plants consistently had smaller roots in relation to tops than non-flowering plants. In our 1945 plantings the most floriferous of the tetraploids had roots slightly below average size, but the control diploids

differed, the plants with the most buds being larger than average. However, the number of diploids for which data were available was not great, some of the most prolific of the bud producers were sterile, and there was also considerable disbudding. The 1946 plants were allowed to produce seed and a more positive trend became apparent. In Tables IV and VI it will be noted that flowering declined in the larger plants, and, in the potted tetraploids of

TABLE VI

RELATIONSHIP BETWEEN FRESH ROOT WEIGHT AND FLOWERING IN FIRST-YEAR, POTTED PLANTS

Series	Type	No. of plants	Av. root weight in grams		
			Flowering plants	Non-flowering plants	All plants
<i>A</i> (moved outside)	$2n$	58	25.8	38.4	26.9
	$4n$	79	21.0	35.0	31.2
<i>B</i> (kept in greenhouse)	$2n$	74	9.7	10.8	10.1
	$4n$	89	13.5	16.9	16.4

Series *A* and *B*, none of the plants with roots exceeding 40 gm. flowered. Among the various  $F_1$  families of tetraploids grown in the garden plot from two-year-old seed, 32.5% of plants with roots under 40 gm. flowered and only 13.5% of those with roots exceeding 40 gm. On the whole it would seem that plants that mature late and flower sparsely if at all during the first year tend to have the largest roots. When flowering and seed production do occur they appear to have an adverse effect on root enlargement.

### Comparison of Diploids and Tetraploids

A striking feature of tetraploids, as contrasted to diploids, was the more robust nature of their organs. The leaves were usually noticeably wider, the average width in mature rosettes being about 50% greater than in diploids (Fig. 3). This greater width, which was so apparent to the eye, created a false impression as to leaf area. Actually the increase in leaf width was negated by a decided reduction in leaf number, so that  $4n$  leaf area generally fell somewhat below that of contemporaneous diploids (Fig. 4). During the early stages of growth tetraploids sometimes exhibited a minor superiority in leaf area, and occasionally again in late summer because of a prolongation of the period of growth and a less pronounced dormancy, but in none of the parallel series of potted plants where estimates were made did the leaf area of the fully developed rosette in tetraploids exceed that in diploids. In this connection it might be noted that the estimates of leaf area were calculated from the number, average length, and width of the leaves with correction for shape and degree of lobing.

In both diploids and tetraploids the number of leaves increased from the first to the second year, and was greater in field than in potted plants. The largest of the second-year field tetraploids had up to 200 leaves, rosettes to 22 in. in diameter, and produced as many as 150 inflorescences in the spring period of blossoming. In all parallel series, however, the diploids had many more leaves than tetraploids. To accommodate the profusion of leaves the diploids more often possessed multiple crowns than the tetraploids.

As in the case of the leaves the tetraploid capitula were generally larger than the diploid, but productivity was drastically reduced. In the various parallel series of first-year diploids and tetraploids grown under similar conditions from year-old untreated seed,  $4n$  head production was only  $1/10$  to  $1/4$  that of diploids. This difference was narrowed in the second year, when some of the tetraploids flowered profusely. As shown by comparison of the 1945 and 1946 plantings of Crosses  $86 \times 71$  and  $71 \times 86$  (Table II) ageing of the seed for another year caused a marked diminution in flowering. This was noted in both diploids and tetraploids.

The root systems of diploids and tetraploids exhibited the same fundamental differences in organization as the aerial parts. The tetraploids tended to have thicker less divided roots, though individuals were highly variable as illustrated in Fig. 6. Both unbranched tap roots and finely divided fibrous systems with many prominent secondaries occurred in tetraploids and diploids, but stout tap roots were more common among the tetraploids and diffuse fibrous systems more prevalent among the diploids. The bearing of these

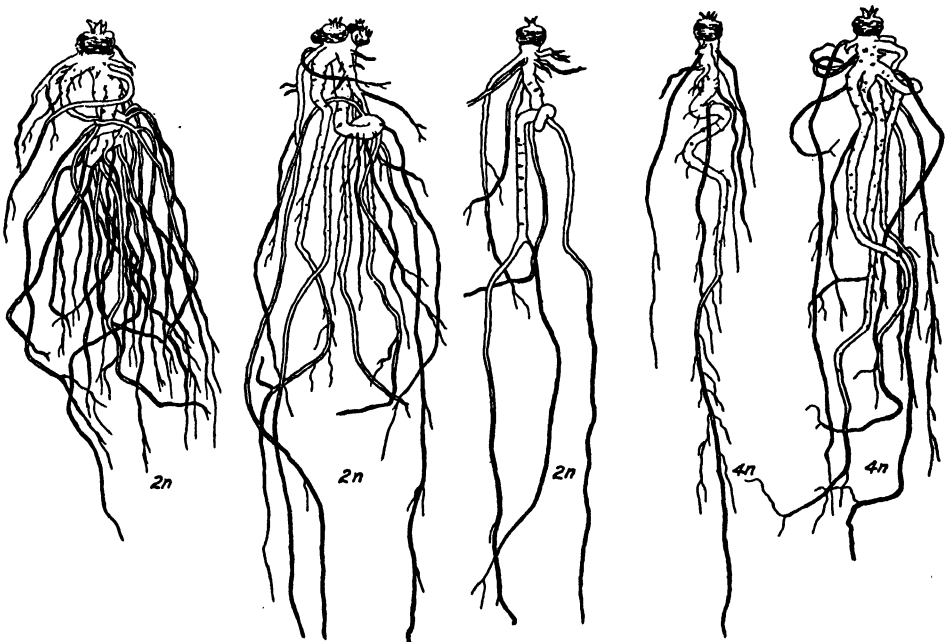


FIG. 6. Types of root systems in first-year diploids and tetraploids. From left to right weights were 38, 48, 33, 23, and 45 gm. Mag.  $\times 1/6$ .

basic differences in root habit on such problems as removal of roots from the soil and extraction of rubber should be recognized when contrasting diploids and tetraploids.

Although the tetraploid roots were inclined to be more robust than the diploid, the increase in thickness was counteracted by reduction in number of divisions so that little difference in total weight was observed between comparable plants. For instance, in the first-year plants of Series A (Table VI) it will be noted that the average root weight of flowering diploids and tetraploids corresponded, being 25.8 and 21.0 gm., respectively. Similarly in the non-flowering plants of the same series the  $2n$  and  $4n$  roots were much alike though substantially heavier at 38.4 and 35.0 gm. When floriferous first-year diploids were disbudded to make them comparable with sparsely flowering tetraploids, their root weight approximated that of the latter. On the other hand, when second-year diploids were disbudded and the more profusely flowering second-year tetraploids allowed to set seed the latter plants developed definitely inferior roots. It would appear from these data that flower and seed production have an inhibiting effect on root formation. Cognizance must be taken of this relationship when contrasting diploids and tetraploids. Since the production of  $4n$  heads during the first year is much lower and the proportion of non-flowering plants higher than in diploids, the average root weight of the tetraploids tends to surpass that of the diploids, unless the latter are disbudded. From the standpoint of root production tetraploids may prove economically superior to diploids because of their sparse first-year flowering.

It should perhaps be emphasized that the seed used in our experiments was stored at room temperature, and was not chilled prior to germination. Certain Russian investigators (Krotkov (5) ) found that exposure of diploid seeds to temperatures of  $0^{\circ}$  to  $2^{\circ}$  C. before germination hastened flowering. Borthwick *et al.* (3) also reported that plants that received low temperatures during their early development flowered more abundantly than untreated plants. If, as appears to be indicated by the data accumulating from various sources, flower and seed production hinder root formation, it would seem advisable to avoid pregermination chilling or other practices that stimulate flowering.

Ageing of the seed for another year still further reduced the flowering of first-year plants. This was most clearly demonstrated by the 1946 and 1945 plantings in pots of the  $F_1$  progenies of  $71 \times 86$  and  $86 \times 71$  (Table II). Flowering was virtually eliminated in the 1946 plants derived from the same seed lots as used in 1945. Tetraploids set out in the garden plot were more floriferous but here again blooming was much reduced as compared with plants of the previous year. Likewise among a small lot of 31 random potted diploids grown from two-year-old seed only three plants flowered. Effect of age of the seed on vegetative growth was not accurately determinable because of the lack of control series. Growth of the tetraploids did not seem impaired, but there were some indications of a slight relative reduction in vigour among the diploids.

## Discussion

When large or small tetraploid kok-saghyz plants were crossed among themselves they did not bear achenes reflecting the difference in stature of the parents, nor did achene size appear to be an important factor in determining ultimate size of derivative plants when these were grown separately. However, since the early growth of seedlings derived from large achenes sometimes exceeded that of seedlings grown from small achenes, especially in the case of the diploids, achene size may be an important factor when competition exists in close planting.

Mature tetraploids differed from diploids in possessing larger but fewer divisions of the plant body, increase in size being counteracted by reduction in number. For instance, leaf width was approximately 50% greater in tetraploids than in diploids, but owing to the greatly curtailed rate of leaf production the leaf area of tetraploids failed to exceed that of diploids in any of the parallel series where estimates were made. The general statement of Navashin *et al.* (7) that tetraploid kok-saghyz was distinguished from the diploid by an increase of all the cells, organs, and the plant as a whole was not confirmed in so far as it relates to the plant as a whole. Whether the differing conclusions are to be ascribed to the fact that Navashin's observations were based on field plants and the present author's largely on potted plants is not clear. The 1945 plantings gave some indication that favourable field conditions might be more conducive to the development of a superiority among the tetraploids than growth in pots, but the number of control, potted plants was too small for adequate comparison.

During the early stages  $4n$  seedlings were more robust than  $2n$  seedlings and leaf area was slightly greater. After the first month, however, the leaf area of tetraploids tended to fall slightly behind that of diploids. As a rule the tetraploids matured shortly after the diploids, the attainment of maximum size of the rosette and the inception of flowering occurring a few days later. Without providing statistics, Navashin *et al.* (7) reported no difference in the proportion of  $4n$  and  $2n$  plants blooming in the first year. This was definitely not the case in the Toronto plantings. The tetraploids were notably less floriferous than diploids, both in the proportion of flowering plants and head productivity per plant. Again the differing observations may be partially explicable by dissimilar seed treatments and conditions of growth. The seed used locally was not given pregermination chilling since such treatment was not considered generally beneficial.

On the whole, plants that possessed broad leafy rosettes, matured late, and flowered sparsely if at all during the first year tended to have the largest roots, though some individuals deviated from the general trend. When flowering and seed production occurred they appeared to have a retarding influence on root enlargement. Since the tetraploids were less floriferous than diploids they were correspondingly freed from the inhibiting effects of flower and seed development, and in that respect had an advantage over diploids. Thus



under certain combinations of internal and environmental conditions tetraploids would be expected to produce larger roots than diploids. That the degree of such superiority fluctuates with the various factors affecting growth is attested by the differing observations of Navashin *et al.* (7), Warmke (11), and Bannan (2).

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## ERYSIPHE GRAMINIS IN CANADA<sup>1</sup>

BY MARGARET NEWTON<sup>2</sup> AND W. J. CHEREWICK<sup>3</sup>

### Abstract

A study has been made of powdery mildew (*Erysiphe graminis* DC.) in Canada, including its distribution, the physiologic specialization of its two varieties, *Hordei* and *Tritici*, and the reactions of barley and wheat hosts to the prevalent races of the two varieties of mildew. The fungus occurs throughout Canada but it is most prevalent in British Columbia, Ontario, and Quebec. Nine races of *E. graminis Hordei* and three of *E. graminis Tritici* have been identified. Races 3, 6, 8, and 9 of the former, and races 4 and 5 of the latter are the most common in Canada. At a higher temperature (25° C.) practically all the races of mildew developed a more resistant type of infection than at a lower temperature (15° C.). The only exception was race 6 of *E. graminis Hordei*, which thrived fairly well at the higher temperature.

The reactions of 246 varieties and strains of barley to nine races of barley mildew and the reactions of 124 wheat varieties and strains to two races of wheat mildew indicate that there are varieties of both cereals that are resistant to powdery mildew.

Powdery mildew, *Erysiphe graminis* DC., is present on wheat, oats, barley, rye, and many grasses in Canada. How far back in the history of Canadian agriculture mildew became established on cereals is not known. The first collections of mildew in Canada were apparently those of Dearness,\* in 1891, on a grass at London, Ont. and, in 1898, on fall wheat at Granton, Ont. Salmon (15), in 1900, reported the presence of powdery mildew in Ontario, and Fraser (5), in 1909, stated that, in Nova Scotia, *E. graminis* "occasionally causes serious damage to grain crops". In 1910, Dimitriou (4, pp. 16-19) reported mildew as abundant and destructive on certain varieties of wheat, barley, and rye in Quebec. It was not until 1921, when the plant disease survey was started in Canada, that systematic records of mildew were made in all parts of Canada. From 1921 to 1945, these records show that powdery mildew was present in Canada every year during that 25-year period, with the possible exception of 1924 when no survey was carried out. It has occurred in every province of Canada, as well as in the North West Territories (11), where, in 1940, the senior author found it on wild grasses along the Mackenzie River as far north as Arctic Red River, approximately 65 miles north of the Arctic Circle.

Powdery mildew constitutes a definite hazard in cereal production in certain parts of Canada (2; 3; 4, pp. 16-19). No specific experiments have been carried out to appraise the actual yearly damage from powdery mildew on

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\* Dates of collections were kindly supplied by Dr. John Dearness. The collection made in 1891 was reported, in 1893, by Ellis and Everhart (N. Am. Fungi 2813 on leaves of grass at London, Ont. J. Dearness).

cereals but reports have shown that definite losses have occurred. In British Columbia, in 1936, Foster\* estimated the damage from powdery mildew on the wheat variety Little Club to be 87% and on the oat variety, Bond, 20%. Reports from Ontario and Quebec refer to barley mildew as the most destructive disease of barley, and wheat mildew as causing considerable damage on winter varieties. A report from Quebec (2\*\*) states, "Winter wheat was severely infected by powdery mildew—80% of the plants were infected, and, at maturity, the heads of these were only half-filled". In the Prairie Provinces, oat mildew has not been reported. Wheat and barley mildew are less prevalent there than in the other provinces and frequently develop too late to bring about material losses. In certain years in the Prairie Provinces (3), however, mildew has been destructive both on barley and on wheat, particularly in localized areas.

Because of the prevalence of wheat and barley mildew in certain parts of Canada, considerable attention has been given to the problem of controlling this disease by the development of resistant varieties. Before breeding programs could be effectively carried out, information was necessary on the occurrence and geographical distribution of physiologic races of wheat and of barley mildew, the environmental conditions governing the spread of these races, and the relative resistance of varieties to them. It was with a view to securing this information for the plant breeders that the present investigation was undertaken in 1934.

### Specialization of *Erysiphe graminis*

That powdery mildew of cereals and grasses is specialized into varieties, "Formes spécialisées", was first demonstrated by Marchal (10) in 1902. He distinguished seven physiologic varieties of mildew: *Triticici* on species of *Triticum*; *Hordei* on species of *Hordeum*; *Secalis* on species of *Secale*; *Avenae* on species of *Avena*; *Poae* on species of *Poa*; *Agropyri* on species of *Agropyron*; and *Bromi* on species of *Bromus*. Homma (6), 27 years later, reported an additional variety of mildew: *Elymi* on *Elymus mollis*.

The significance of Marchal's discovery of specialization stimulated further research, and Salmon (16) in Europe and Reed (12) in the United States made extensive tests with mildew on grasses and cereals. They confirmed much of Marchal's work and, in addition, found that the varieties described by him were not necessarily the ultimate units of specialization. Salmon found evidence that the mildew on *Bromus* spp. could be subdivided into four or possibly five physiologic races—"biologic forms"—and Reed showed that the mildew on *Poa* spp. consisted of "more than one at least partially differentiated physiologic form".

It remained for Mains and his co-workers to prove that specialization also existed in *E. graminis* attacking cereals. In 1930, Mains and Dietz (9)

\* Unpublished data by W. R. Foster, Provincial Department of Agriculture, Victoria, B.C.

\*\*Twelfth Annual Report of the Canadian Plant Disease Survey, p. 7. 1932.

described five physiologic races of *E. graminis Hordei* Marchal in the United States and, in 1937, Tidd (18) reported two additional races in that country. In Germany, in 1934, Honecker (7) identified nine physiologic races of mildew but, owing to the fact that he used but one of the differential hosts used by Mains and Dietz and other North American workers, his races cannot be compared with those isolated in America. In Canada, four races of barley mildew were reported in 1935 (2\*) and three in 1944 (3). Since 1944, two additional races have been isolated, making a total of nine physiologic races isolated in Canada.

Specialization within the variety *E. graminis Tritici* Marchal was first established by Mains (8). From 1923 to 1933, he tested the reactions of a large number of wheat varieties to mildew, and by the reactions of the varieties Axminster and Norka established two pathogenically distinct races. In Germany, Rosenstiel (14), in 1938, found evidence of specialization in this organism, and Schlichtling (17), in 1939, isolated six distinct races that, apparently, were all different from the two recognized by Mains. In the same year, Waterhouse (20) reported three races in Australia, and Vallega (19), in 1941, three in South America. In Canada, the present authors isolated three physiologic races of *E. graminis Tritici*. As the investigators outside North America worked independently of each other, their respective physiologic races were identified by means of different groups of differential hosts with the result that the races of *E. graminis Tritici* in different countries are not necessarily comparable.

## Identification of Physiologic Races of *Erysiphe graminis*

### THE DIFFERENTIAL VARIETIES

The differential varieties of barley used in Canada in the identification of physiologic races of barley mildew were the same as those used by Mains and his co-workers (9, 18), with the addition of Chevron C.I. 1111 (3). The wheat varieties, used in the identification of wheat mildew, were the same as those suggested by Mains (8). Owing to the fact that different selections of the same agronomic variety of a cereal may react quite differently to certain races of mildew (8, 9), the seed of most of the differential hosts was originally obtained from Mains and subsequently attempts were made to keep it as pure as possible.

### SOURCE OF CULTURES

Cultures were established from both conidia and perithecia. Because of the fact that, at temperatures above freezing, conidia of *E. graminis* germinate within 24 hr. (3) very few cultures were established from conidia taken from specimens sent through the mail. Cultures were secured quite often, however, from specimens that had been four to five days in transit when the infected plants were packed loosely in a cardboard box or cylinder with the roots or cut ends wrapped in moist material so as to maintain the specimens in a living

\* *Fifteenth Annual Report of the Canadian Plant Disease Survey*, p. 13. 1935.

condition. Such specimens, when received, were usually devoid of viable conidia, but fresh conidia developed within 24 hr. upon floating the infected leaves on a 10% sucrose solution.

A number of cultures were established from perithecial material sent through the mail in ordinary paper envelopes. Such material has been kept by the writers for three years in a refrigerator at approximately 3° C., with no appreciable loss of viability. After it was shown (3) that alternating temperatures induce perithecial development, and that perithecia alternately wetted and dried develop ascospores, the pure cultures of physiologic races were frequently stored from season to season in the perithecial stage.

### INOCULATION METHODS

Host seedlings were grown in 4-in. pots with from 20 to 30 seedlings per pot. The seedlings in the pots were protected from stray mildew spores by glass chimneys with inverted Petri dishes placed on top of them or by being kept in special isolation booths with glass on three sides, glass or cellophane on top, and cotton curtains in front.

The seedlings were inoculated when the first leaf was about 4 in. long. When conidia were used as inoculum to establish a culture, they were applied to the surface of a leaf by means of an inoculating needle with a spatulate metal tip, or by drawing a conidium-bearing specimen over the leaf to be inoculated. Since conidia germinate at a relatively low humidity (1, 3, 21), the inoculated plants were left in the isolation booths without incubation in moist chambers. When perithecia were used to start a culture, the specimen was first soaked in water until ascospores began to form. It was then fixed on a moist filter paper in a Petri dish and this was placed on top of a glass chimney over the seedlings for 24 to 48 hr. Transpiration by the seedlings was usually sufficient to keep the filter paper damp.

When a culture had been established seedlings of the differential hosts were inoculated with it by shaking over them, within the isolation booth, a pot of heavily infected seedlings bearing an abundance of viable conidia.

### THE ISOLATION OF PURE RACES FROM MIXED CULTURES

It was a common experience when field cultures of either conidial or perithecial origin were studied to find that two or more physiologic races were present in the same culture. A number of these races could be detected by a casual inspection of the reactions of the differential hosts. For example, a mixture of races 1 and 3 of *E. graminis Hordei* would result in two types of infection, "1" and "4", on Black Hull-less, Nepal, and Peruvian barley seedlings (see Table II). In such instances a separation of races was effected by making single-pustule isolations from both types of infection and then transferring the conidia to susceptible hosts in separate isolation booths. Other races, such as 10 and 3, when mixed were more difficult to separate and, therefore, single-pustule isolations through several generations of the organism were often necessary. In certain cases, however, single-spore isolations were

made before the identity of the race was definitely established. The identity of a new race was never finally established until several single-spore isolations had been made and their infection types on the differential hosts had been compared with those of the known races.

#### THE EFFECT OF TEMPERATURE ON THE DETERMINATION OF PHYSIOLOGIC RACES OF *Erysiphe graminis*

Difficulties have been experienced by the writers, as well as by other investigators (3, 13), in maintaining cultures of powdery mildew in the greenhouse during the summer months. Still greater difficulties were encountered in the determination of physiologic races during such periods. Races identified in the spring, when again tested during the summer months, usually produced considerably weaker infections. Temperature appeared to be the main limiting factor and, therefore, a number of tests were made to determine the effect of temperature on the development of powdery mildew.

Duplicate sets of seedlings of the differential hosts of wheat and barley were inoculated separately with each of six races of *E. graminis Hordei* and one race of *E. graminis Tritici* and were placed in two adjacent compartments of a temperature-controlled greenhouse. In one compartment the mean temperature was held at approximately 25° C., and in the other at approximately 15° C. These temperatures were chosen after it was found by preliminary tests that the development of mildew was completely arrested when the host plant was kept continuously at a temperature above 25° C. On the other hand, development was very slow when the host plant was kept continuously below 15° C. The tests were repeated three times.

The average infection types produced by six physiologic races of *E. graminis Hordei* on six differential hosts are summarized in Table I. The infection types of the one race of *E. graminis Tritici*, that was included in the test, were affected by the two different temperatures in the same way as those of *E. graminis Hordei*.

Although the effect of temperature varied slightly with different races on different hosts (Table I), the development of mildew in general was appreciably less at the higher temperature. The infection types shown in Table I were recorded at the time of maximum development of the disease; that is, six to eight days after inoculation in the hot house (25° C.) and 11 to 14 days after inoculation in the cold house (15° C.). Two to three days after the maximum development had been reached at the higher temperature, mildew pustules ceased to produce conidia and turned into a resistant type of infection with thin mycelial mats. There was little, if any, secondary infection. The only exception was in the case of race 6 of *E. graminis Hordei*, which thrived fairly well at the mean temperature of 25° C., a fact indicating that not all the races of mildew are equally responsive to temperature changes. At the lower temperature both wheat and barley mildew pustules continued to sporulate, with an abundant development of secondary infections, until the host seedlings were killed.

TABLE I

MEAN INFECTION TYPES<sup>1</sup> PRODUCED BY SIX PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei* ON SEEDLING LEAVES OF SIX BARLEY VARIETIES AT TWO DIFFERENT TEMPERATURES

Host	Physiologic races											
	3		4		6		8		9		10	
	Temperature, ° C.											
	25°	15°	25°	15°	25°	15°	25°	15°	25°	15°	25°	15°
Black Hull-less	3—	3+	3	4	1	1+	3	4—	2—	1	3—	2
Chevron	;	1—	0	0	1—	1—	3—	4	3—	4—	1	1—
Goldfoil	0	0	;	1—	0	0	0	0	0	0	0	0
Heil's Hanna	3+	4	4—	4+	4	4—	4—	4+	3+	4	4—	4+
Nepal	3	4	3+	4	2—	1+	3—	4—	2+	2—	3	2—
Peruvian	2	3+	1—	1+	1—	1	1	1	1+	1	3—	3

<sup>1</sup>Explanation of symbols

0—immune—no visible signs of infection.

;—highly resistant—chlorotic or necrotic flecks present with no visible development of mycelium.

1—highly resistant—with a slight development of mycelium.

2—moderately resistant—moderate development of mycelium with a slight production of conidia. Chlorotic or necrotic spots present.

3—moderately susceptible—moderate to abundant development of mycelium accompanied by moderate sporulation. Chlorotic or necrotic spots may be present.

4—very susceptible—large pustules abundantly sporulating. No necrotic spots.

(+) and (—) signs indicate quantitative increase or decrease in the infection type.

The responsiveness of mildew infections to temperature changes emphasizes the importance of identifying physiologic races, as far as possible, under conditions of constant temperature. Owing to the fact that identification work has to be done in greenhouses where temperatures may run above 25° C., particularly during late spring and summer months, a knowledge of the way in which temperature influences mildew reactions is important, and makes it possible to gauge to some extent the modifications brought about by variations in temperature.

Studies reported in this paper, on both physiologic specialization and the reactions of varieties and strains of barley and wheat to powdery mildew, were made in a greenhouse maintained at a mean temperature of approximately 20° C.

### Physiologic Races of *Erysiphe graminis Hordei*

During the first five years of the survey (1934 to 1939) races 1, 3, 4, and 6 were isolated (Table II). These four races resemble one another in their inability to infect Chevron (C.I. 1111), a barley that in Canada, prior to 1940, proved to be highly resistant to both mildew and stem rust and was used

Analytical key for the identification of physiologic races of *Erysiphe graminis Hordei* on the basis of their pathogenicity on six selected differential varieties of barley.

Goldfoil susceptible	
Black Hull-less susceptible	5
Black Hull-less resistant	
Chevron susceptible	12
Chevron resistant	7
Goldfoil resistant	
Chevron susceptible	
Nepal susceptible	
Peruvian susceptible	11
Peruvian resistant	8
Nepal resistant	9
Chevron resistant	
Peruvian susceptible	
Nepal susceptible	3
Nepal resistant	10
Peruvian resistant	
Black Hull-less susceptible	4
Black Hull-less resistant	
Nepal susceptible	2
Nepal resistant	
Heil's Hanna susceptible	6
Heil's Hanna resistant	1

TABLE II

MEAN INFECTION TYPES PRODUCED ON SEEDLING LEAVES OF SIX VARIETIES OF BARLEY BY 12 PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*

Physiologic race	Black Hull-less C.I. 666	Chevron C.I. 1111	Goldfoil C.I. 928	Heil's Hanna 3 C.I. 682	Nepal C.I. 595	Peruvian C.I. 935
1	0-2	0-;	0	1-2	1-2	0-1
2*	1-2	0-1	0	4	4	1
3	3	0-1	0	4	4	3-4
4	4	0-;	0-;	3-4	4	1
5*	3	-	4	4	4	4
6	0-2	0-1	0	3-4	0-1	0-1
7*	0-1	0-;	3-4	3-4	0-1	0-1
8	3	3-4	0	3-4	3-4	0-1
9	1-2	4	0	4	1-2	0-1
10	1-2	0-1	0	4	1-2	3
11	4	4	0	4	4	4
12	1	3	4	4	1-2	1

\* Races not isolated by writers.

quite extensively in breeding work. In that year, P. R. Cowan, Cercalst, Central Experimental Farm, Ottawa, reported that, in his plots, Chevron barley was heavily infected by mildew. Subsequent studies showed that the mildew collected by him on Chevron barley could not be distinguished from race 4 except by the reaction of Chevron; and this made it advisable to add Chevron to the list of five differentials selected by Mains and his colleagues (9, 18) for the identification of physiologic races of barley mildew.





Between 1940 and 1945, notable changes took place in the common races (Table III). Five new races were identified, of which four attacked Chevron, namely, races 8, 9, 11, and 12. These four races comprised 50% of the isolates during that period, and they already form a potential threat to newly developed barley varieties with the Chevron type of resistance.

Two of the races that attack Chevron, races 8 and 9, together with races 3 and 6, constitute the prevalent races in Canada.

Races 10 and 11 were isolated only from perithecial cultures. The former was isolated four times from perithecia collected in Manitoba, and the latter from perithecia sent from Ontario and British Columbia.

Races 2, 5, and 7 identified in the United States (9, 18), have not been found in Canada.

The annual distribution of the nine physiologic races of *E. graminis Hordei* isolated in Canada from 1934 to 1945, races 1, 3, 4, 6, 8, 9, 10, 11, and 12, is shown in Table III.

### Physiologic Races of *Erysiphe graminis Tritici*

Annual surveys of the prevalence of physiologic races of wheat mildew, *E. graminis Tritici*, have not been conducted in Canada but, from 1933 to 1944, a total of 27 cultures from British Columbia, Alberta, Manitoba, Ontario, and Quebec were studied.

Until 1943, only one physiologic race was identified. This race, isolated in Manitoba, attacked the wheat varieties Huron and Chul, but not Norka and

TABLE IV

MEAN INFECTION TYPES PRODUCED ON SEEDLING LEAVES OF FOUR VARIETIES OF WHEAT BY FIVE PHYSIOLOGIC RACES OF *Erysiphe graminis Tritici*

Races 1 and 2 were isolated in the United States by Dr. E. B. Mains.

Races 3, 4, and 5 were isolated in Canada by the present authors.

Race	Axminster R.L. 75	Chul R.L. 543	Huron R.L. 20	Norka R.L. 1888
1 (United States)	0	0	0-1	0
2 (United States)	0	0	0	4
3 (Canada)	0	4	4	0
4 (Canada)	0	0	4	0
5 (Canada)	4	0	4	4

Analytical key for the identification of physiologic races of *Erysiphe graminis Tritici* on the basis of their pathogenicity on four selected differential varieties of wheat.

Axminster susceptible	5
Axminster resistant	
Chul susceptible	3
Chul resistant	
Norka susceptible	
Huron susceptible	4
Huron resistant	2
Norka resistant	1

Axminster (Table IV). In 1943 seven cultures from Manitoba were studied. They attacked the variety Huron but not Chul, Norka, and Axminster. In 1944, a culture that attacked Huron, Norka, and Axminster but not Chul (Table IV) was obtained from an Ontario specimen. The infection types of the three Canadian races, as well as those of races 1 and 2, the first to be isolated in America (8) are included in Table IV. A study of these infection types makes it clear that the three races identified in Canada are all different from the two described by Mains, and that they should be designated as new races. They are tentatively numbered races 3, 4, and 5 (Canada). A key for the identification of the five races found in North America is included with Table IV.

### **Relative Resistance of Barley and Wheat Varieties to Powdery Mildew**

A study of the relative resistance of varieties and strains of barley and wheat to physiologic races of powdery mildew was undertaken chiefly to assist plant breeders in Canada in choosing suitable breeding material and in developing resistant varieties.

These tests were made in the greenhouse where each variety could be studied for its reaction to individual races of mildew. Such tests are considered to be more reliable than field tests because powdery mildew does not develop to the same extent year after year in any one district, nor do all races occur in a district at the same time. In the greenhouse also, the reactions of varieties in the seedling stage may be determined quickly and accurately. Since all the varieties tested by the authors and found to be resistant to mildew in the seedling stage have also proved to be resistant in the adult stage, it should be possible to select resistant varieties on the basis of seedling tests. There are some varieties, however, in which the seedlings and adult plants do not react identically to powdery mildew. Some variation in varietal reaction to powdery mildew has been shown by the writers to be due to environmental conditions, particularly temperature, but even under similar conditions, some varieties are more resistant in the adult than in the seedling stage. Such varieties possess so-called adult plant resistance.

Tidd (18), in his study of races 6 and 7 of *E. graminis Hordei*, obtained a greater degree of adult plant resistance in the spring than during the winter. He mentions, however, that the temperature in the spring went up to 32.2° C. while in the winter it ranged between 12.7° and 24° C. In the tests made by the authors it was found that no host that was completely susceptible in the seedling stage showed complete resistance in the adult stage. Nevertheless, a number of varieties, particularly of durum wheat, possess a high degree of adult plant resistance (Table V).

Varieties of cereals possessing the adult plant type of resistance (Table V) might be expected to have considerable value as breeding material, but a still better starting point for a breeding programme, having as its object the development of mildew resistant varieties, would be to choose as parental

TABLE V

A COMPARISON OF THE INFECTION TYPES ON SEEDLING AND ADULT PLANTS OF FIVE VARIETIES EACH OF WHEAT AND BARLEY INOCULATED WITH *Erysiphe graminis* *Tritici* RACE 4 AND *E. graminis Hordei* RACE 3

Variety	Accession No.	Infection types	
		Seedlings	Adult plants
Wheat			
Gaza	R.L. 1664	4	1+
Kanred	" 1443	4—	2+
Mindum	" 568	4	1+
Pentad	" 203	4	1+
Thatcher	" 1945	4	4
Barley			
Blackhull	C.I. 878	4	2
Black Hull-less	" 666	4	2+
Bolivia	" 1257	2	0
Flynn	" 1311	3	2
Heil's Hanna	" 682	4	4

material suitable varieties known to be immune or highly resistant in the seedling stage.

Many of the varieties tested in the present investigation have been developed recently, but for purposes of comparison some of the older standard varieties have been included as well. All the varieties of barley tested are listed in Table VI. Those having Canadian accession numbers are placed first; those with United States cereal introduction numbers last.

As pointed out elsewhere in this paper, in most cases varieties develop increased resistance to mildew at higher temperatures. In the present greenhouse tests, special precautions were taken to prevent the temperatures from becoming excessively high at any time.

TABLE VI

INFECTION TYPES ON 246 BARLEY VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE WITH NINE PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*

Variety or strain	C.A. No.	Infection types with physiologic races								
		1	3	4	6	8	9	10	11	12
<i>Hordeum vulgare</i>										
Aethiops	892	1	2+	2	1	2—	2	2+	1	1
Algerian	10	—	0	;	;	0	;	0	1=	;
Arlington Awnless	882	—	—	;	;	—	1—	;	—	1—
Atlas	702	2	4	1—	2	1	1	4	3—	1
Atrum	888	1	2—	2	2—	2+	1+	2	1—	2—
Baker	87	—	—	4	3	—	4	4	4	4
Bark	703	4—	4	4	4	4	4	4	4	4
Bay Brewing	707	4	4	4	4	4	3	4	—	1
Beecher	1153	—	3+	4—	4	4	4—	4	3	1

TABLE VI—Continued

INFECTION TYPES ON 246 BARLEY VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
WITH NINE PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*—Continued

Variety or strain	C.A. No.	Infection types with physiologic races								
		1	3	4	6	8	9	10	11	12
<i>Hordeum vulgare</i> —										
Continued										
Beldi Giant	1024	2+	3+	2+	3+	4	2+	3	4	2
Black Barbless	11	3	4	3	4	4	4	2+	2+	4
Black Hull-less	761	2+	4	4	1+	4	1	1+	4	2
Blue Hull-less	760	3	4	4	2+	4	1+	1	4-	1
Bolivia	12	—	1+	1+	3+	1	1+	2+	1+	1-
Byng	1096	—	4	4	4	4	4	4	4	4
California Brewing	706	—	4	4	4	4	4	4	4	4
California Feed	26	—	3	4-	4	4	3+	4	4	4
California Mariout	1083	4-	4	4	4	4	4	4	4	4
Cape	708	—	4	4	4	3+	3+	4	4	3
Chevron	1121	—	1-	;	1	3+	4-	1-	3	4
Chillian Brewing	709	—	2+	3	3+	1	2+	4	1	3-
Club Mariout	729	3-	4	4	4	4	4	4	3+	3-
Coast	13	4	4	4	4	4	4	4	4	4
Coast	711	—	3	4	3-	4	4	—	—	—
Coerulescens	17	—	2	1+	2+	2+	1+	4	1	1=
Colsess	772	2	3	1	1+	2	1	4	2+	1
Danubian	1020	4	4	4	4	4	4	4	4	4
Duplex	1129	—	0	1-	0	;	1+	;	1+	2
Eurylepis	15	—	4	4	4	4	4	4	4-	4
Featherston	715	4	4	4	4	4	4	4	4	4
Flynn	1032	3	3	1-	1+	1	1-	4	1-	1-
Galore	1126	—	4	4	4	4	4	4	—	4
Galore × Duplex	(Guelph)	—	;	;	;	;	1-	;	;	1-
Garton	1134	4	4	4	4	4	4	4	4	4
Gatami	717	2+	2	2	2-	1+	2+	2-	4	1
Glabron	718	4	4	4	4	4	4	4	4	4
Hero	719	3	4	4	4	4	4	4	4	4-
Horsford	777	4	4	4	4	4	4	4	4	4
Horsford	84	—	3	4	3	3+	3-	4	4	3-
Horsfordianum	887	4	4	4	4	4	4	4	4	4
Icelandic	90	—	4	4	4	4	4	4	4	4
Juliaca	43	—	3	4	4	4-	4	4-	2	4
Kindred	1155	—	4	4	3	4	4	3+	4	4
Lico	1152	—	4	3	3	2+	4	4	2	1
Lion	86	4	4	4	3+	4	4	4	4	4
Malting	92	—	4	3+	4	4	4	4	4	4
Manchuria	724	4	4	4	4	4	4	4	4	4
Manchurian	726	4	4	4	4	4	4	4	3	4
Mansfield	1056	4	4	4	3	4	4	4	4	4
Mariout B113	91	—	2+	4	4-	3	4	3	4	2-
Mariout B	1130	—	2+	2+	3+	2+	2+	—	—	—
Mensury	730	—	4	4	4	4	4	4	3+	4
Mensury Ott. 60 ×										
Duplex	79	—	1+	1+	1-	0	1	1-	1-	1
Mensury Ott. 60 ×										
Duplex	80	—	0	0	0	;	1	0	;	1
Mensury Ott. 60 ×										
Duplex	81	—	0	;	0	1-	1-	0	1-	1=
Michigan Black	28	4	4	4	4	4	4	4	3	4
Minsturdy	732	1=	2+	1	1+	1-	1	1	1+	1+
Montcalm	1135	—	4	4	4	4	4	4	4	4
Morocco	1131	—	4	1	1+	1	1-	4	2+	1-
Nepal	779	2+	4	4	1	4	2+	1	4	1
Newal	1089	—	4	4	4	4	4	4	4	4



TABLE VI—Continued

INFECTION TYPES ON 246 BARLEY VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
WITH NINE PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*—Continued

Variety or strain	C.A. No.	Infection types with physiologic races								
		1	3	4	6	8	9	10	11	12
<i>Hordeum vulgare</i> — Continued										
Telli	751	4	4	4	4	4	4—	4	4	—
Texan	1173	—	4—	4—	4—	4	3	3+	2+	1+
Titan	1118	—	1—	2+	2	1—	3+	3	4	2
Trebi	753	4	4	4	4	4	4	4	4	4
Tregal	1150	—	4	4	4	4	4	4	4	4
Trifurcatum	891	2	4	4	1+	4	1	1+	2+	2—
Vaughn	759	3+	4	4	4	4	4	4	3	2+
Velvet	755	4	4	4	4	4	4	4	3+	4
Velvet	1133	—	2+	4	3+	2+	4	4	3	—
(Velvet × Olli) × (Peatland × Duplex)	74	—	;	1—	1	;	1+	0	1	1
(Velvet × Olli) (Peatland × Duplex)	75	—	0	0	0	1—	1	0	1—	;
(Velvet × Olli) × (Peatland × Duplex)	76	—	1=	;	0	1—	1—	;	1=	1
(Velvet × Olli) × (Peatland × Duplex)	77	—	0	0	0	1	1	0	1—	1
(Velvet × Olli) × (Peatland × Duplex)	78	—	1—	1+	;	1+	1+	1	1—	1—
Velvon	1151	—	4	4	4	4	4	4	4	4
Virginia Hooded	39	—	3+	4	4	4	4	4	4	4
Warrior	1144	—	4	4—	4	4	4	4—	4	4
White Gatami	40	—	1—	1+	2	1—	1+	1	1+	1
White Hull-less	785	1+	4	4	1	3+	1	1	4	1
Wisconsin Sel.	42	4	4	4	4	4	4	4	4	4
Wisconsin 38	1101	4	4	4	4	4—	3+	4	4	4
<i>Hordeum distichon</i>										
Alpha	801	4	4	4	4—	4	4	4	—	4
Angustispicatum	900	2	1	1+	2+	2+	1	2	1	1+
Archer	88	—	4	4	3	4	4	4	—	4
Archer Goldthorpe	1003	4	4	4	4	4	4	4	4	4
Austrian Hanna St. 66	46	—	4	1	1+	2+	1+	4—	1	1
Baku	870	2	4	3—	3+	4	3	2+	2	2
Canadian Thorpe	816	—	4	4	4	4	4	4	4	4
Charlottetown 80	1100	3	4	4	4	4	4	4	4	4
Chevalier	83	3—	4	4	4	4	4	4	4	4
Clifford	825	4	4	4	4	4	4	4	—	4
Danish Island	1002	3+	4	4	4	4	4	4	3	4
Duckbill	826	4	4	4	4	4	4	4	4	4
Frankonia	1017	2	3+	1+	2+	1+	1	4	1+	1
French Chevalier	822	4	4	4	4	4	4	4	4	4
Foreign 127	48	—	3—	1+	2+	3	1+	4	2	1+
German Brewing	1008	4	4	4	4	3+	4—	4	4	2+
Gold	829	4	4	4	4	4	4	4	4	3
Golden Drop	49	4—	4	4	4	4	4	4	4	4
Golden Pheasant	830	4	4	4	4	4	4	4	4	4
Goldfoil	50	;	0	1—	0	0	;	0	0	4
Gordon	833	4	4	4	4	4	4	4	4	4
Halikon	52	1+	3—	1+	1+	1+	1	2+	1	1—
Halikon	834	1	4—	1+	1+	4	1+	3—	1+	1+
Hanna P3	53	4	4	4	4	4	4	4	4	4—
Hanna	63	—	0	0	;	0	0	0	0	4
Hanna	64	—	4	4	4	4	4	4	4	2—

TABLE VI—Continued

 INFECTION TYPES ON 246 BARLEY VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
 WITH NINE PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*—Continued

Variety or strain	C.A. No.	Infection types with physiologic races									
		1	3	4	6	8	9	10	11	12	
<i>Hordeum distichon</i> —											
Concluded											
Hannchen	837	4	4	4	4	4	4	4	3+	2+	
Heil's Hanna 3	61	—	4	4	4	4	4	4	4	4	
Horn	1078	4—	4	4	4	4	4	4	4	4	
Italy	54	2+	1+	3—	3	4	3—	2+	2+	2	
Michigan 2-row	55	4	4	4	4	4	4	4	4	4	
Nigrilaxum	788	2+	3+	2+	1+	2+	2	2+	2	2—	
Nigrinudum	787	2—	1	1	1	1—	1	1	1—	2—	
Nigrum	21	3	0	;	1	4	4	1	2+	4	
Nudum	22	2+	2+	1+	2+	2	2+	2+	2	2—	
Nutans	23	—	3+	1+	3—	2—	1+	4	1+	1=	
Nutans A	24	—	4	4	4	4	4	4	3+	4	
Nutans K	25	—	4	4	4	4	4	4	4	4	
Orel	852	—	4	4	4	4	4	4	4	4	
Orel	14	1+	1=	1	1=	2	1+	1	2—	1+	
Plumage Archer	1004	4	4	4	4	4	4	4	4	4	
Polish	56	4	4	4—	4	4	4—	4	4	1	
Princess	57	4—	4	4	4	4	4	4	4	4	
Prussian	58	1+	4—	2	3—	2+	1+	4	2+	1	
Rex	1113	—	4	4	4	4	4	4	4	4	
Rimpai	786	2+	1+	2+	3	3	2	2+	1	2+	
Sanalta	1088	—	4	4	4	4	4	4	4	4	
Scotch Standwell	1007	4—	4	4	4	4	4	4	4	4	
Smyrna	859	4	4	4	4	4	4	4	—	4	
Smyrna	1052	—	3+	4	3+	4	4	4	4	4	
Spartan	860	4	4	3+	4—	4	4	4	—	4	
Steigum	862	—	4	4	4	4	4	4	4	4	
Stephan	1142	—	;	;	;	;	;	0	;	4	
Svalof	59	4	4	4	4	4	4	4	4	2	
Svalof Victory	868	4	4	4	4	4	4	4	4	2	
Svanhals	864	4	4	4	4	4	4	4	—	4	
Svansota	865	4	4	4	4	4	4—	4	—	4	
Wheeler's Thorpe	60	4	3+	4	4	4	4	4	3	4	
<i>Hordeum deficiens</i>											
Blackhull	813	2	4	1	1	1+	1=	3+	1	1+	
Decorticatum	881	2	1	1	3—	1+	1	2+	1—	1	
Deficiens	880	3	4	4—	4	4—	3—	4	1+	3—	
Foreign 82B	62	—	2—	2—	2	2+	1+	2	1	1—	
Steudelli	789	1+	1+	1+	1	1	1	1+	1	1+	
Triceros	790	4	4	4	4	4	4—	4	3	3	
Tridax	791	2	2	1+	2	2+	1+	2	1	2—	
<i>Hordeum intermedium</i>											
Cornutum	897	2	4	4—	2	3	2—	2	2+	1+	
Mortoni	894	3	1	1—	2—	3	1+	4	2	2	
Nudihaxtoni	895	2+	4	4	4	4	4	4	3	4	
Nudimortoni	896	4—	4	4	4	4	4	4	4	4	



TABLE VI—*Concluded*

INFECTION TYPES ON 246 BARLEY VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
WITH NINE PHYSIOLOGIC RACES OF *Erysiphe graminis Hordei*—*Concluded*

Name and description <sup>1</sup>	C.I. No.	Infection types with physiologic races							
		3	4	6	8	9	10	11	12
Beldi	190	4	4	4	4	4	4	4	4
Coast	276	2—	3—	1+	1+	3—	1	1+	1—
Coast	510	4	4	4	4	4	4	—	4
Himalaya	620	2	1	1	1	1—	4—	3	1
Hooded Spring H	716	4	4	4	4	4	4	4	4
Oderbrucker	957	4	4	4	4	4	4	4	4
Luth	972	2	1—	0	1+	1	3	1	1—
Kwan	1016	3+	2	;	;	1+	1=	1	1—
Abacus	1088	3	2+	1—	;	1	1—	1+	1
	1347	4	4	4	4	4	4	—	4
Abyssinia	2192	4	;	1—	1+	1—	4	1—	1=
Purple Nepal PHN	2242	4	4	1—	4	3	1	4	1—
Arequipa	2329	3—	1—	1	2+	1	4	1+	1=
Callas	2440	4	4	4	4	4	4	4	4
Modia	2483	;	;	0	;	;	;	0	0
	2538	1+	2+	3	2	3—	1	3	2—
	2542	4	4	4	3+	4	4	3+	2+
	2549	3	4	4	2+	4	1+	3+	2+
Carre 26	3386	1+	;	;	;	1=	1=	1	;
Carre 180	3390	2+	3	3	3+	4	2+	3	2
Batna	3391	4	1	1=	1=	1—	4	1	1—
Mianwali	3400	0	0	0	0	;	;	0	0
Palmella Blue 2SS	3609	0	;	0	0	;	;	0	1—
	3737	2+	1—	;	;	1—	1=	1—	1—
Sahara	3770	4	2	2	2	2—	4	2	2
Morocco	3902.1	1+	;	0	0	1—	2	1=	;
	4156.2	0	0	0	1+	4	0	3+	2—
	4160.1	;	1=	0	;	1+	;	1+	1
	4219	0	;	;	;	;	0	0	;
	4220.1	0	;	;	0	;	0	0	1=
	4223.2	1	2	2	1+	2—	1	1	2—
	4356	0	1—	1=	;	1=	;	0	1
	4974	0	0	0	0	0	1=	0	1=
	4975	1+	1—	0	;	1	2—	1—	;
	4979	0	0	0	0	0	0	0	0
Paso S	5047	2+	2—	2+	2	3	2	2	2
	5326	4—	4	;	4	2+	1=	4	1—
	5366	4	4	1=	4	1	1	4	1
	5644	0	;	0	0	1	0	0	1=
	5647	0	0	0	;	1+	;	0	;
	5862	0	;	0	0	;	;	0	;
	5863	0	;	0	0	;	;	0	;
Peruvian Sel. 1	5912	1	;	0	0	1—	1	0	1=
	6015	;	1	;	;	1—	1=	;	1=
Cebada Capa	6193	1=	1=	0	0	;	1	1—	1=
	6306	0	0	0	0	;	0	0	0
Morocco	6311	1+	;	0	0	;	1	0	;
Peruvian Sel. 19	6568	1	1—	0	0	1—	1	0	1—

<sup>1</sup> All varieties are *Hordeum vulgare*, rough-awned, white or blue, with adhering lemma and palea, except where otherwise indicated as follows:

2 = 2-rowed.

H = hooded.

N = naked kernels.

P = purple.

S = smooth awned.

SS = semi-smooth awned.

The races of barley mildew used in the inoculation tests include all the races so far isolated in Canada; those of wheat mildew include two out of the three races isolated in Canada. Two pots of seedlings, with approximately 30 seedlings to a pot, were inoculated with each physiologic race. With some varieties a considerable number of tests had to be made to establish a satisfactory mean, although with the majority a single test only sufficed. In Tables VI and VII only the average of all the reactions of each variety is indicated.

TABLE VII

INFECTION TYPES ON 124 WHEAT VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE WITH TWO PHYSIOLOGIC RACES OF *Erysiphe graminis Tritici*

Variety or strain	Station No.	Infection types with physiologic races	
		4	5
<i>Triticum vulgare</i>			
Apex	R.L. 1342	4	3
Apex × S-615	Swift Current 4188	4	4
Axminster	R.L. 75	1—	4
Brevit	" 1890	4	3
CAN 1926	" 1948	1	4
Canus	" 1321	4	3—
Carina	" 1889	4	3
Ceres	" 127	4	4
Chinese × Emmer	" 1802	3—	2—
Chinese × Marquis	" 1596	4	3
Chinese × Progress	" 1804	3	4
Chinese × Progress	" 1805	4	1—
Chul	" 543	0	0
Coronation	" 729	1—	0
Democrat	" 1535	4	4
Double Cross	" 2303	3+	4
Double Cross	" 2315	4—	4
D.C. × Ceres	" 625	4	4—
(D.C. × H44) × Marquis	U. Sask. A-31-1	4—	—
(D.C. × H44) × Marquis	U. Sask. A-35-1	4—	—
Early Triumph	R.L. 94	4	4
Eureka	" 1534	4	—
Federation	" 1395	4	4
Fultz	" 1539	4	4
Fultz Sel.	" 1540	4	4
Garnet	" 15	4—	3
Glenwood	" 1411	4	4
Hard Federation	" 1887	4	4
Haynes Blue Stem	" 200	4	3
Hope	" 209	1	0
Hope × Marquis	C-26-86-12	3—	—
Hope × Reliance	R.L. 1110	1—	—
Hope × Reward	" 1165	3—	1—
Hope × Reward	" 1165.2	2—	1—
Hope × Reward	" 1165.3	2—	1—
Hope × Reward	" 1165.4	4—	;
Hope × Reward	" 1165.5	2—	—
Hope × Reward	" 1165.6	3	—
Huron	" 20	4	4
Hussar	" 1892	4—	—
H44-24	" 229	1—	1—
H44-24 × Marquis	" 704.1	1—	1—
H44-24 × Marquis	" 1081	1—	1

TABLE VII—Continued

INFECTION TYPES ON 124 WHEAT VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
WITH TWO PHYSIOLOGIC RACES OF *Erysiphe graminis Triticici*—Continued

Variety or strain	Station No.	Infection types with physiologic races	
		1	2
<i>Triticum vulgare</i> —Continued			
H44-24 × Reward	" 967	1	1
H44-24 × Reward	" 1097	1	1+
H44-24 × Reward	" 1150	1	2
H44-24 × Thatcher	R.L. 1946	1—	1—
H44-24 × Thatcher	" 1947	1—	0
Illinois No. 1B8	" 1593	3—	4
Kenya	" 1373	4	4
Kenya	" 1374	4	4
Kenya	" 1375	4	4
Kenya	" 1376	4	4
Kenya	" 1377	4	4
Kanred	" 1443	4—	4
Kawvale	" 1451	4	4
Kota	" 571	4	4
K33	" 1885	4	4
Liguleless	" 1936	4—	4
Loros	" 1891	4	4
Malakof	" 1538	4	4
Marquillo	" 132	4	4
Marquis	" 84	4—	4
Ma. × (H44-24-Ma.)	" 1333	1—	0
Ma. × Iumillo	" 1544	4	4
Ma. × Kanred	" 52	4	4—
Ma. × (Pentad × Ma.)	" 1326	4	4
McMurachy	" 1313	4	4—
Mediterranean	" 1537	4	3—
Merit	" 1949	1	0
Minor	" 2058	4	4
NA 95	" 2063	4	3
Norka	" 1888	0	4
Pentad × Marquis	" 986	2+	—
Pentad × Marquis	M.A.C. 12-10-3	4—	—
Pentad × Marquis	M.A.C. 19-19-3	4—	—
Pilot	R.L. 1822	1	1
Prelude	" 25	4	4
Preston	" 207	1	4
Quality	" 133	4	4
Red Bobs	" 1827	4	4
Red Egyptian	" 2061	4	4
Red Fife	" 22	4	4
Redman	" 1834	1+	1—
Red Thatcher	" 1812	4	4
Regent	" 975.1	1	0
Regent	" 975.6	1—	0
Regent	" 975.11	1—	;
Reliance	" 198	3—	4
Renfrew	" 135	4	4
Renown	" 716.6	1	2—
Reward	" 79	4	4
Ridit	" 1932	4—	4
Rival	" 1494	4	4
R.L. 625 × Mercury	" 2054	4	4
R.L. 625 × Mercury	" 2055	4	4
R.L. 704 × Marquis	" 1527	2—	1—
Thatcher	" 1945	4	4

TABLE VII—Concluded

 INFECTION TYPES ON 124 WHEAT VARIETIES AND STRAINS INOCULATED IN THE SEEDLING STAGE  
 WITH TWO PHYSIOLOGIC RACES OF *Erysiphe graminis Tritici*—Concluded

Variety or strain	Station No.	Infection types with physiologic races	
		1	2
<i>Triticum vulgare</i> —Concluded			
Thatcher × Regent	" 1829	1	1
Thew	" 1883	0	4
Warden × Hybrid English	" 1803	4	4—
Webster	" 365	4	4
<i>Triticum durum</i>			
Acme	R.L. 566	4—	4
Akrona	" 1252	4	4
Arnautka	" 570	4	4
Belaturka	" 1412	2—	;
Carleton	" 1663	2+	3
Gaza	" 1664	4	4
Golden Ball	" 1256	4	4
Iumillo	" 7	4	4
Iumillo × Mindum	" 1183	4	4
Kubanka	" 565	4	4
Mindum	" 568	4	4
Pelissier	" 145	4	4
Pentad	" 203	4	4
Spelmar	" 569	4	4
Stewart	" 1683	4	4
Tibet	" 1396	4	4
<i>Triticum compactum</i>			
Jenkin	R.L. 1814	4	4
Little Club	" 223	4	4
<i>Triticum dicoccum</i>			
Black Persian	R.L. 388	;	0
Khapli	" 563	;	0
Vernal	" 567	2—	1
<i>Triticum monococcum</i>			
Einkorn	R.L. 227	1—	1—
<i>Triticum timopheevi</i>	" 1308	0	0

## Results

The varietal reactions to powdery mildew, as shown in Tables VI and VII, indicate that there is no lack of suitable mildew-resistant breeding material in either barley or wheat. In both of these cereals there exist varieties that are highly resistant to all of the physiologic races of mildew found in Canada. In the case of barley, there are resistant varieties in all but one species tested, *Hordeum intermedium*. In the *vulgare* species, 44 varieties, including 16 Duplex hybrids, are immune or highly resistant to the Canadian races of barley mildew. In *H. distichon* nine, and in *H. deficiens* three varieties possess the same high resistance to all the races. Some of the resistant varieties, such as Duplex (C.A.N. 1129), Psaknon (C.A.N. 34), and Minsturdy

(C.A.N. 732) have also been reported (9, 18) as being resistant to races isolated in the United States but not as yet found in Canada.

Similar results were obtained with wheat. Of the *Triticum vulgare* wheats tested, Chul (R.L. 543), Hope (R.L. 209), H-44-24 (R.L. 229), and most of the varieties and strains derived from crosses having Hope or H-44-24 as one parent, showed very high resistance to the two physiologic races employed in these tests. A variety Kanred (R.L. 1443) was completely susceptible in the seedling stage but acquired moderate resistance in the adult stage (Table V). In *T. durum*, only one variety, Belaturka (R.L. 1412), showed high resistance in the seedling stage to both races, but three other varieties, Gaza (R.L. 1664), Mindum (R.L. 568), and Pentad (R.L. 203), although susceptible in the seedling stage, developed resistance in the adult stage (Table V). The varieties of *T. dicoccum*, *T. monococcum*, and *T. timopheevi* that were tested all proved to be resistant in the seedling stage to the two races.

An interesting feature made evident by Table VI is the fact that all hybrids containing the highly resistant parent, Duplex (C.A.N. 1129), proved very resistant to all the known Canadian races of *E. graminis Hordei*. Apparently resistance in that variety was dominant. Similarly, many wheat hybrids derived from either Hope or H-44-24 were resistant to the two races of *E. graminis Tritici* (Table VII).

It appears, therefore, that it should be possible without great difficulty to combine in these two cereals resistance to powdery mildew with other desirable characters. In fact, the varieties of hard red spring wheat recently developed in Canada, such as Coronation (R.L. 729), Regent (R.L. 975), and Redman (R.L. 1884), are resistant to the presently known Canadian races of *E. graminis Tritici*.

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## THE SEASONAL VARIATION IN THE ASCORBIC ACID CONTENT OF EDIBLE WILD PLANTS COMMONLY FOUND IN NEW BRUNSWICK<sup>1</sup>

BY ELIZABETH A. BAIRD<sup>2</sup> AND MURIEL G. LANE<sup>3</sup>

### Abstract

The ascorbic acid content in the leaves of 10 wild plants that grow in New Brunswick was determined at approximately two-week intervals from June to September. 'Free' and 'total' dehydroascorbic acid were measured, using the method of Roe. The total ascorbic acid is highest in young green leaves. After the plants have flowered the ascorbic acid decreases. It becomes almost negligible when the leaves have wilted and turned brown. 'Free' dehydroascorbic acid was present in all 10 plants in small quantity. It fluctuated from time to time but remained fairly constant for all the plants during the period of investigation, and was independent of the 'total' value for that plant. The values for ascorbic acid have been measured for ripe strawberries, for fiddle-heads, and for several common vegetables over the same summer months. They have been tabulated for comparison with the values of ascorbic acid of the 10 wild plants.

### Introduction

A survey was made during the summer of 1946 of the seasonal variation of ascorbic acid in the leaves of 10 different wild plants that grow in New Brunswick.

The plants that were tested were:

1. *Amaranthus retroflexus* L.—amaranthus.
2. *Capsella Bursa-pastoris* (L.) Medic.—shepherd's purse.
3. *Chenopodium album* L.—lamb's quarters.
4. *Oxalis montana* Raf.—wood sorrel.
5. *Portulaca oleracea* L.—portulaca.
6. *Rumex Acetosella* L.—sheep sorrel.
7. *Rumex crispus* L.—curled dock.
8. *Sedum purpureum* Tausch.—live-forever.
9. *Stellaria media* (L.) Cyrill.—chickweed.
10. *Tragopogon pratensis* L.—goat's beard.

These plants were chosen following a preliminary survey during the previous season when the ascorbic acid, carotene, and riboflavin had been determined

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for about 70 plants. They had also been tested for palatability. These 10 plants rated high in the palatability tests and contained moderate to high amounts of ascorbic acid, carotene, and riboflavin (1). Thus it appears that while most of them are classed as weeds, they can now be recommended highly for use on the table, some raw, as salad greens, and some cooked, as spinach. Indeed the vitamin content of many of these plants was much higher than that of many of the vegetables commonly used today.

### Methods

The method for estimating ascorbic acid was that of Roe and Oesterling (3). Both 'free' and 'total' dehydroascorbic acid were determined following the original method, that is, with the dinitrophenylhydrazine reagent and incubation for three hours at 37° C. It should be noted here that this method measures only dehydroascorbic acid. However, by means of a second determination, reduced ascorbic acid can be measured also, due to the fact that it may be converted to dehydroascorbic acid by addition of Norit to a metaphosphoric acid extract. The total dehydroascorbic acid then present is determined in the usual way, and the reduced ascorbic acid may be obtained by subtraction of the 'free' dehydroascorbic from the 'total' dehydroascorbic acid. The terms 'free' and 'total' dehydroascorbic acid, therefore, are used in this paper rather than reduced ascorbic acid and dehydroascorbic acid.

The percentage dry weight was obtained for each sample by drying duplicate samples to constant weight in a drying oven at 95° to 100° C., usually about two days.

### Sampling

All the species were common in this area. They were all good to eat so as to be of practical value. Some were small plants and some were large; some had heavy foliage and some had very few leaves. They came from different habitats, all within a reasonably small area but from patches that were typical for each species. Some were high in ascorbic acid and some were lower. They represented nine different families. Each plot was observed from early spring and the plants were tested as soon as they became available in sufficient quantity. Plants of the same species were obtained from the same patch to eliminate the effect of soils and microclimate on the ascorbic acid content.

The plants were obtained at approximately two-week intervals starting from the time each species made its first appearance in the spring. The samples were all obtained fresh on the morning of the day on which they were analysed.

Analysis was done on the edible portion; as a rule, only the leaves were used, except when the stems were small and tender, and could be served as an integral part of the green, as in the case of wood sorrel or chickweed.

The leaves were separated from the plants and torn into small pieces by hand. The use of a steel knife was avoided to prevent possible oxidation of ascorbic acid by the metal. After being mixed with the metaphosphoric acid,

they were ground in a Waring Blendor. The leaves from several plants were well mixed to get a representative sample. A 5 to 10 gm. sample was used for each determination.

The vegetables, the fiddleheads, and the strawberries were bought fresh from stores or at the market.

## Results

Figs. 1A and 2A show in graphic form the values for 'total' dehydroascorbic acid for the 10 different plants at different dates throughout the season. The values are in milligrams per 100 gm. of dry weight. They were calculated from the fresh weight values and the percentage dry weights.

Many of the plants showed considerable fluctuation of ascorbic acid with fresh weight values, but as the percentage dry weight varied with the weather conditions it was felt that the dry weight values expressed the trend of the seasonal variation better than the fresh weight values.

Figs. 1B and 2B show similarly the values for 'free' dehydroascorbic acid, obtained at the same time as the 'total' values.

Fig. 3 represents the values for 'total' dehydroascorbic acid for strawberries, for fiddleheads, and for several common vegetables, some of which were tested during the summer of 1945 and some during 1946. It has been included for comparative purposes to show the relative values of certain well known vegetables. It has been drawn to the same scale as the previous figures so the graphs for the vegetables and wild plants are comparable.

## Discussion of Results

Examination of the results reveals that goat's beard, shepherd's purse, lamb's quarters, and amaranthus (Fig. 1A) all gave the highest values for 'total' dehydroascorbic acid the first time they were examined after the plants had flowered. The flowers were first observed on these plants on different dates, namely, June 10, July 8, July 18, and July 31, respectively. Live-forever showed a steady decrease in ascorbic acid content. Only once, on July 29, were flowering plants examined. The flowering plants gave a slightly higher value than the two previous tests, but in general this species showed a steady decrease.

Five other plants, namely curled dock, wood sorrel, sheep sorrel, portulaca, and chickweed (Fig. 2A) all gave their highest values for the 'total' estimation made just before flowering, followed by a slow but steady decrease. Flowers were first reported on these plants on June 11, June 13, June 20, July 3, and July 18 respectively, and the highest values were obtained two weeks previous to these dates.

The 'total' dehydroascorbic acid varied considerably with different plants but the tendency for all plants was to decrease after flowering.

The plants were not examined daily for the first appearance of flowers. It seems safe to assume, however, that the two-week period marking the

beginning of flowering represents the period of maximum ascorbic acid content for the 10 plants studied. No attempt has been made to correlate the appearance of flowers with ascorbic acid content. Probably both are manifestations of maturity that may or may not be independent of each other.

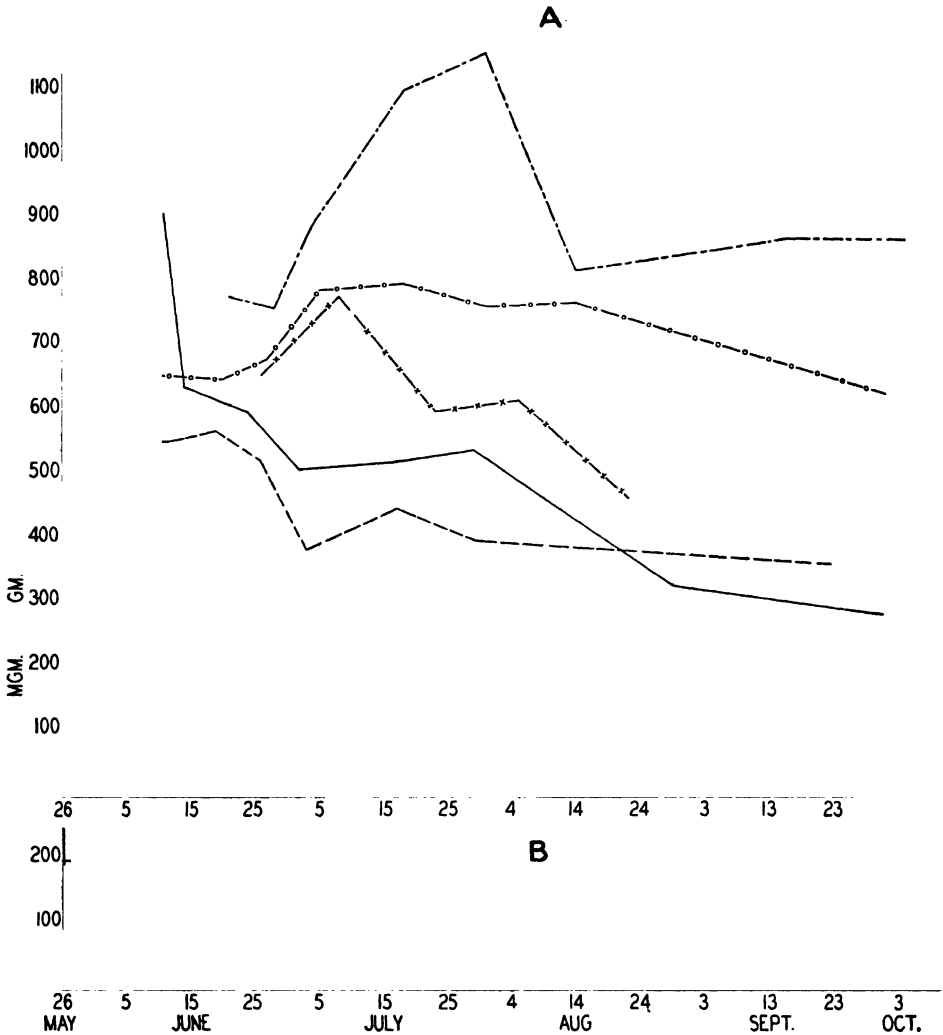


FIG. 1. Seasonal variation in the ascorbic acid content of live-forever, goat's beard, amaranthus, lamb's quarters, and shepherd's purse. A—'total' ascorbic acid. B—'free' dehydroascorbic acid.

The values for 'free' dehydroascorbic acid (Figs. 1 B and 2 B) showed much scattering, for all the plants tested. There was little variation between species and even within species no seasonal trend could be observed.

The ratio of 'free' to 'total' dehydroascorbic acid varied from 10 to 25%. This is in agreement with Tuba *et al.* (4) who found that in green plants,

such as pigweed, cabbage, and peas, dehydroascorbic acid exceeded 10% of the ascorbic acid value. The ratio is slightly higher than that found by Roe and Oesterling (3) who reported the dehydroascorbic acid in some green plants to be about 5% of the amount of ascorbic acid.

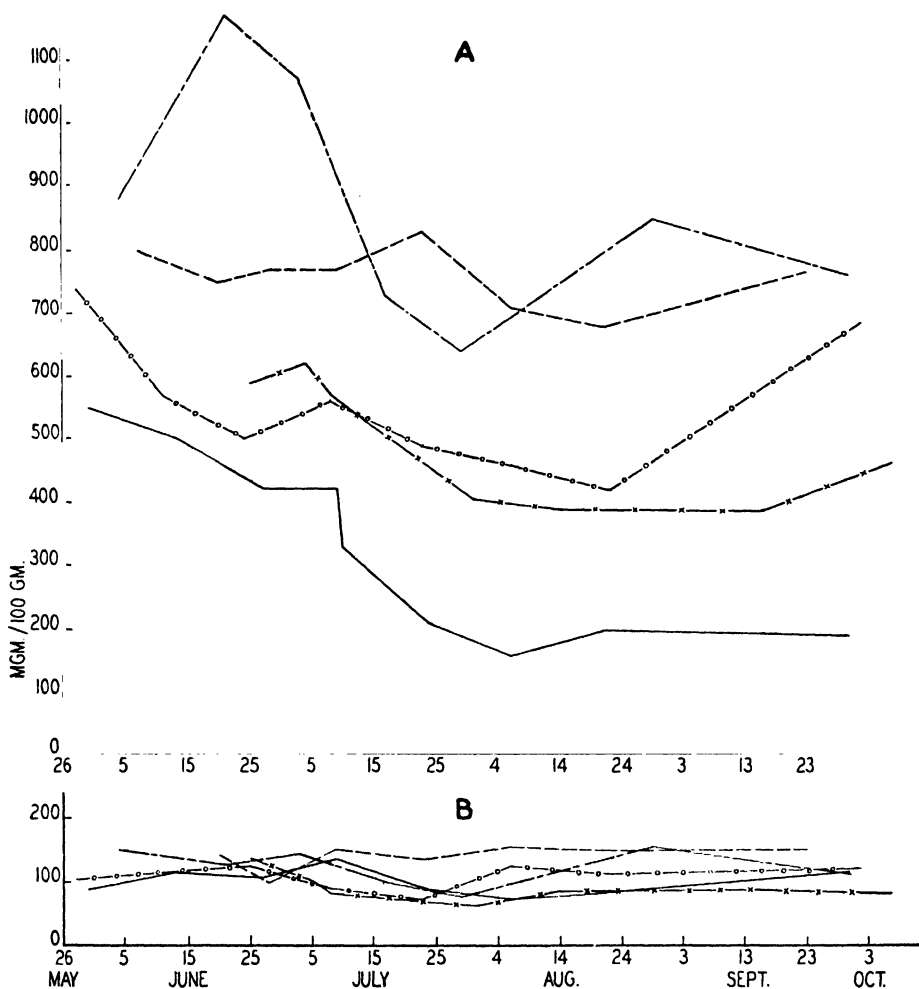


FIG. 2. Seasonal variation in the ascorbic acid content of sheep sorrel, wood sorrel, portulaca, curled dock, and chickweed. A—'total' ascorbic acid. B—'free' dehydroascorbic acid.

The dock and the wood sorrel gave slightly higher values than the other plants for 'free' dehydroascorbic acid. They were both highly pigmented after extraction and although blanks were run to make allowance for the colour effect, these two remained noticeably higher than the others. The 'total' extracts, after treatment with Norit, were always colourless.

Although the tendency was for the ascorbic acid to decrease after flowering it must be borne in mind that this was a survey of wild plants from their

natural environment. Hence it was impossible to state the exact age of each individual plant.

For curled dock, for example, the first five samples were obtained from the same patch. By Aug. 29 all these plants had completely died down, so the two last samples were taken from another plot nearby that was known to have started growing later in the season. These two samples, which were still fresh and green when tested, showed an increase over the final samples from the first plot. A sample of shepherd's purse, picked on Aug. 5, that contained plants that had died down and were almost completely brown, gave a very low value of 200 mgm. per 100 gm. It was not included in Fig. 1 A as

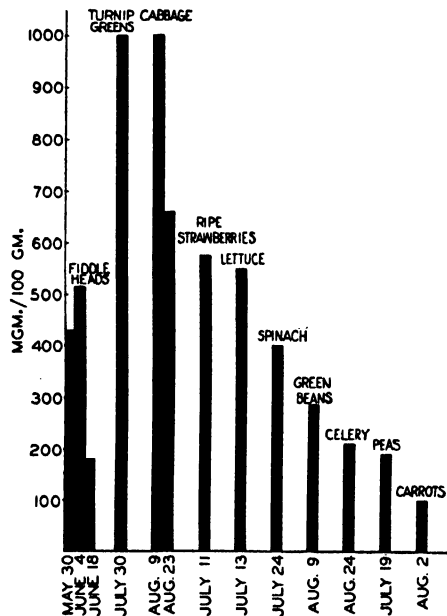


FIG. 3. 'Total' ascorbic acid content of several common vegetables.

we planned to test only representative samples at different seasons. In the case of wood sorrel the seasonal variation was slight; these plants always appeared to be at about the same stage of development with new flowers appearing constantly.

In view of these observations, the variation would appear to depend on the stage of development. It almost coincided with seasonal variation so that the higher values are obtained early in the summer. However, plants of the same species at the same stage of development, whether early or late, gave approximately the same values for ascorbic acid.

Finally, it appears that the ascorbic acid values of these wild plants compare favourably with those of several common vegetables (Fig. 3). Amaranthus and curled dock, at their best, gave higher values than cabbage or turnip greens. The values found in lamb's quarters, shepherd's purse, wood sorrel,

sheep sorrel, portulaca, and goat's beard were lower than those in cabbage but in the same range as those of lettuce or strawberries and higher than those of spinach or green beans. The amounts in chickweed and live-forever, the two wild plants in which values were lowest, were higher than in celery, peas, or carrots.

This is in agreement with the results of Murray and Stratton (2), who found values for dock to be twice as high as for spinach; shepherd's purse and lamb's quarters about equal to spinach; and chickweed considerably lower.

Fiddleheads, *Onoclea Struthiopteris*, which are commonly used along the Saint John River valley in early spring as a cooked green, contained a moderate amount of ascorbic acid. Like the other plants studied, the amount of 'total' dehydroascorbic acid decreased at the end of their season.

These 10 representatives of the edible wild plants of New Brunswick could be used as a valuable dietary source of ascorbic acid. From the study of the seasonal variation it became evident that they can be most highly recommended for use in the early summer before the garden vegetables become plentiful.

### Acknowledgments

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## MEANS OF INOCULATION OF THE DUTCH ELM DISEASE BY *HYLURGOPINUS RUFIPES* EICHH.<sup>1</sup>

BY RENÉ POMERLEAU<sup>2</sup>

### Abstract

Investigations on the transmission of the Dutch elm disease were carried out during summer of 1946 in Canada, where the only known vector of the fungus is the native bark beetle, *Hylurgopinus rufipes*. By close observations of young elms, recently and naturally infected with *Ceratostomella Ulmi*, it was found that by boring tunnels through the thin bark of twigs or small branches to the cambium, the adults of this beetle can inoculate the disease to healthy trees.

Of the two known insect vectors of *Ceratostomella Ulmi* (Schwarz) Buis. found in the United States (2), only the native beetle, *Hylurgopinus rufipes* Eichh. occurs in Canada and is associated with this outbreak of the Dutch elm disease.

The apparently rapid spread of the disease in Eastern Canada (7), in the absence of the European beetle, *Scolytus multistriatus* Marsh, draws anew attention to the behaviour of the native beetle, although it is not considered as a very effective carrier of the pathogen in the United States (8). Proof has been given that adults of the European bark beetle can transmit the disease through twig crotch feeding injury (4, 6, 9), whereas the means of spread of the pathogen in the field by the native beetle has been incompletely worked out.

Collins *et al.* (3) found that adults of *H. rufipes*, confined in cages, feed on twigs and trunk of young elm in which they bore holes similar to those produced by *S. multistriatus*. Kaston and Riggs (5) have found in Connecticut bark tunnels that they believe the beetle bores for feeding purposes before it constructs brood galleries in dying parts of elms. Yet neither these authors nor others have demonstrated how in nature the insect introduces the fungus inside the bark of healthy trees.

Since 1945 branches of several tall infected felled trees were closely examined with the purpose of finding feeding injuries produced by *H. rufipes* on twigs. As no scars similar to those made by *S. multistriatus* in the United States were found, we have therefore endeavoured to find, through other methods, the usual way of entrance of the spores. The well known habit of the native insect of finding shelter in the bark of elm during fall (1) has long ago suggested to some American scientists that the fungus may come in contact with xylem by this means. In order to check over this opinion, overwintering cells of the beetle were examined in the bark of several trees located in the heavily

<sup>1</sup> Manuscript received March 11, 1947.

Contribution from the Division of Forest Pathology, Department of Lands and Forests, Quebec.

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infected area. It has not been possible in any case to trace in the bark of the trunk or large limbs a tunnel chew inward from the cell to the cambial region before emergence.

In order to obtain information on the transmission of contagion to healthy elms in Canada, young elms, just showing the first symptoms of the disease, were selected in 1946 in the vicinity of the towns of Berthierville and Sorel, the centre of the infected area. Some 30 trees answering to these requirements were found, cut down, and carefully studied. Under the bark of most of them, by following the characteristic brown streaks, it was possible to disclose one or several places where the inoculum was left. These marks are always connected to a small tunnel dug by an insect through thin bark. The puncture left on the sapwood by the insect is shallow and of about 1 mm. in diameter (Fig. 1). In most instances they are surrounded by an elliptical dark spot, measuring  $4-10 \times 3-5$  mm. (Fig. 2). Sometimes, when the infection of the vascular system is in its first stage, only a small, faint, brownish discoloration can be seen around the bite (Fig. 3), or the only trace left on the wood by the close passage of the insect is a tiny brown speck.

Several of these spots are to be seen, on the wood of certain trees, especially when the trees are growing close to dying ones or those heavily infested with *H. rufipes*. The tunnels are sometimes numerous in rough parts of the bark (Fig. 4) or near the axil of a twig (Fig. 5). They are short and extend usually to an emergence hole nearby. It is important to mention here that these small channels dug to the sapwood were noticed only on twigs or limbs less than 2 in. in diameter. Hence, one can readily understand that the bark beetle may more easily reach the living xylem and deposit spores on it through the thin bark of branches of this size than through the thick bark of larger branches or trunk. The bark or feeding tunnels, noticed already by Kaston and Riggs (5), from ground line to small limb on elms in Connecticut, do not differ from those we have described here except, at least under conditions that prevail in Canada, that they are sometimes burrowed to the sapwood.

In few instances, the origin of the infection on twigs can only be traced close to a mechanical injury or a dead part due to sunscald (Fig. 6) or canker, along which an insect has succeeded in boring a tunnel to living wood.

In order to ascertain that all the trees studied were infected, samples of twigs were taken near the point of origin of the disease and kept for culturing in the laboratory. Cultures from all these samples have yielded *C. Ulmi*. The fact that adults of *H. rufipes* were often found in such tunnels does not leave any doubt of their responsibility as vector of the disease.

The results of this investigation, carried out in Canada, where the action of the only known vector of the fungus is not hidden by the injuries of the European *Scolytus*, as in the United States, indicate clearly that *H. rufipes* can readily inoculate *C. Ulmi* to healthy elm by boring up tunnels to sapwood through the thin bark of twigs and small branches.



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FIG. 1. Punctures made by *Hylurgopinus rufipes* on the sapwood of an elm twig.

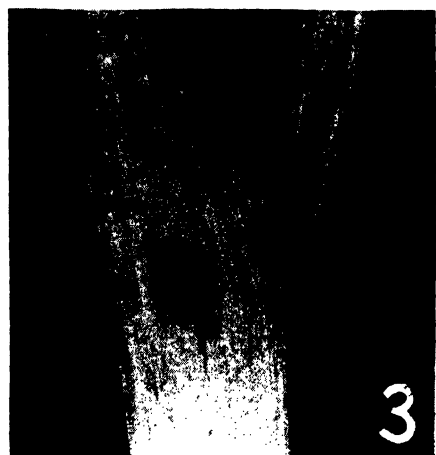
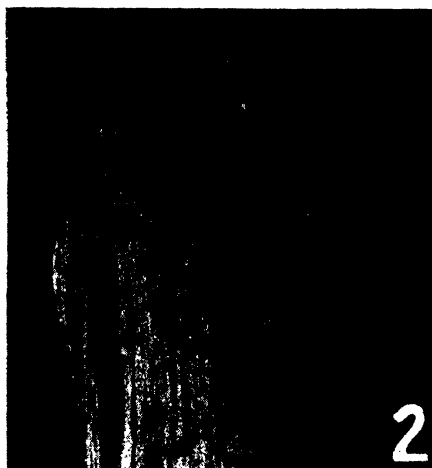
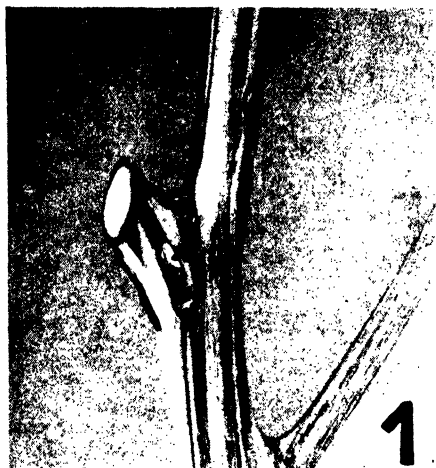
FIG. 2. Dark spots formed around insect injuries on sapwood of an elm twig.

FIG. 3. Faint brownish discoloration around wounds produced by the insect on the sapwood of an elm twig.

FIG. 4. Several tunnels bored by the insect in rough part of the bark of an elm twig.

FIG. 5. Tunnels dug by the insect near the axil of an elm twig.

FIG. 6. Insect puncture on the sapwood near a dead part of an elm twig.





## A STUDY OF THE SPECIES OF *ENTYLOMA* ON NORTH AMERICAN COMPOSITES<sup>1</sup>

BY D. B. O. SAVILE<sup>2</sup>

### Abstract

A study of the species of *Entyloma* on North American composites suggests that almost every form can be assigned to one of two phyletic groups. *E. polysporum* contains most of the forms with densely crowded, rather large teliospores and no conidia. *E. Davisii* on *Rudbeckia hirta* and, probably, a fungus on *Lepachys columnaris* are segregated from it. *E. Compositarum* contains most of the forms with smaller, uncrowded spores and abundant conidia. *E. arnicale* and possibly one or two other forms may be regarded as segregates from this species.

Emended descriptions of *E. polysporum* and *E. Compositarum* are given to clarify the differences between these two species. Corrections are also made in the identification of some exsiccati specimens.

Comparisons are made with European forms to the extent that material has been available, but far more study of European specimens is needed to allow assessment of some species.

Species concepts for these fungi are discussed, and criteria are suggested for adequate morphological studies.

The present study had its origin in the observation that the leaf smut of *Gaillardia aristata* agreed more closely with *Entyloma Compositarum* than with *E. polysporum* to which it had been ascribed. Examination of these two species on a number of hosts revealed that they had been widely confused. This confusion seems to have arisen partly from the misnaming of certain specimens in American exsiccati and partly from the inadequacy of the original descriptions. As has already been indicated (Savile, 13, 14), all available Canadian collections on *Gaillardia* are assignable to *E. Compositarum*.

Before discussing the individual collections upon which this study is based, some consideration of the species concept in *Entyloma* is necessary. American mycologists have probably been more prone than European workers to abide by the morphological species concept for parasitic fungi, where such a concept is workable, and to assign designations below the rank of species to those entities that are distinguishable only by host specialization. It must be admitted that a compromise is often necessary where the basis of morphological distinction is meagre. It may be helpful to consider the method rather widely, if tacitly, adopted in the Uredinales, which are perhaps on a sounder taxonomic basis than any other major parasitic group. In very few instances could much progress be made in the identification of a rust if no information on the identity of the host were available. It is common practice to regard morphologically similar rusts as one species if they occur on closely

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related hosts and as distinct species if the hosts are not closely related. Unquestionably some errors are introduced by such a method, but on the whole it has proved to be workable and accurate.

In the Ustilaginales there are fewer morphological characters available than in the Uredinales, owing to the simpler life cycle. Consequently there is a greater temptation to depart entirely from the morphological basis of classification, and some writers, notably Ciferri (4) and the Sydows (19), have separated species almost entirely on the basis of host identity with little or no evidence even of specialization on that host. The concensus of opinion may eventually demand such a narrow species concept; but, in any event, it is desirable that the fullest use be made of such morphological characters as do exist. The present study has repeatedly shown that this requirement has not been fulfilled, and the discussion to follow will show examples both of inadequate descriptions and of disregard of definite morphological distinctions. Ciferri's (5) discussion of the species concept with special reference to the smut fungi contains a good example of the latter failing in the statement: "Under the caption, *Entyloma Calendulae* (Oud.) de Bary, European mycologists included all *Entyloma* living on the members of the Compositae family. On the other hand, American mycologists assigned the same function to *E. Compositarum* Farl. The two species, from the morphological standpoint, are identical". The early students of this genus actually showed much more discrimination than Ciferri suggests, but the important point is that Ciferri, despite his extensive study of this genus and his erection of several new species on composites in North America (4), apparently failed to observe abundant morphological differences existing among certain of the groups involved. *Entyloma Calendulae* and *E. Compositarum* are almost as dissimilar as any two species of the genus.

In adapting this modified morphological species concept to the group under discussion, no one is more aware than the writer that it is imperfect and that certain arbitrary decisions have to be made. These difficulties have considerably delayed completion of the study. Clearly one cannot rely solely on morphology. For example, *Entyloma Lobeliae*, recently collected locally, was suspected of being *E. Compositarum* until the host developed sufficiently to be recognized as *Lobelia inflata*; yet probably no one would suggest that these species should be united, although it is conceivable that their similar morphology is ascribable to a common origin rather than to chance convergence.

Morphological constancy, sufficient to prevent the masking of small differences between species, is a prerequisite to such a system. Studies in some genera of the Hyphomycetes suggest that variation is so great in many species as to make any approach to a purely morphological classification hopeless. Even this conclusion does not make careful measurement of specimens unnecessary; without it one cannot hope to say how many species actually attack a plant. One must, however, rely rather largely on host relationships for the delimitation of species. Ideally every fungus would be cultured and its host range determined by experiment, but in practice such a

treatment can only be applied to a trifling proportion of our specimens, and one must, in any event, make use of herbarium material that was not subjected to experiment.

In the leaf smuts under consideration it has not been possible to examine adequate material on some hosts; but, where abundant specimens have been available, variability has proved to be so slight that some confidence may be placed in an adequate description.

In considering such a climax group as the Compositae one should recollect that physiological differentiation need not always exactly follow morphological trends. Accordingly the limits of tribes and genera are sometimes indefinite, and parasitic fungi may have a seemingly anomalous host range. Two beliefs concerning the Compositae have to some extent influenced the assignment of specific limits in this study: firstly, that the various tribes of the Tubuliflorae are much more intimately related to each other than to the Liguliflorae; and, secondly, that the segregation of the "Ambrosiaceae" from the Tubuliflorae is not justified. These beliefs stem in part from the relationship of these plants with the fungi that parasitize them.

The characters that have proved most useful in drawing up practical descriptions of these fungi are: minimum and maximum length of teliospores, minimum and maximum width of teliospores, minimum and maximum teliospore wall thickness, density of teliospore distribution, and presence or absence of conidia or conidiophores. The literature abounds with descriptions of spores such as "mostly 12–15 $\mu$  diameter" and "averaging 14 $\mu$  long". In practice, average widths and lengths are very difficult to determine accurately. If the spores were dispersed upon a slide, complete fields could be measured and the danger of unconscious selection would be reduced to a minimum; but in a section there is always a considerable proportion of spores that cannot be clearly viewed, and selection, with its threat of error, is inevitable. If, after measuring a number of randomly selected spores, one searches for extremes of length and width, it will generally be found, after examining two or three sections, that little further enlargement of the range can be secured; and the extremes so found usually agree quite closely with those of other specimens. The maximum length tends to be more variable than other dimensions, owing to the occasional occurrence of distorted elongate spores. Measurement of these spores with relatively constant but small differences should always be made under the oil-immersion objective. Spore wall thickness must be used with discretion. Slight differences tend to occur from host to host, and the walls are generally thicker in *Entyloma polysporum* than in *E. Compositarum*; but the range in a given specimen is generally too great to merit much faith in wall thickness alone, except in outstanding forms such as the fungus on *Rudbeckia hirta*. The approximate wall thicknesses given in many descriptions have no practical value. The density of teliospore distribution has proved to be more constant than might be expected, both in a given host and from one host to another. Conidia may be difficult to find, especially in old specimens, but conidiophores can almost always be found in the stomata in

species that are known to bear conidia; occasionally it is impossible to find conidiophores in old lesions overrun by saprophytic fungi.

Sections are required for adequate examination, but the making of them need not be as tedious as cutting sections in pith demands. Many hours have been saved by the development of a technique of chopping sections under the dissecting microscope (Savile, 16). This method has the further advantage of being economical of material, an important factor in the examination of rare and scanty specimens.

The dimensions of the conidia have not proved to be of much value; for it is often impossible to find enough in a specimen to determine the size range, variability seems to be as great within as between species, and those in a particular specimen may be acicular or falcate according to the weather conditions prior to the time of collection.

One of the characters listed may be atypical in a given specimen, but most collections conform to a particular concept well enough to allow one to assign them fairly confidently to a species.

The appearance of the lesions is generally fairly constant on a given plant, but it may vary widely from host to host. On certain plants that are attacked by more than one species of *Entyloma* it has diagnostic value.

In Table I will be found the characteristics of a large number of North American collections of *Entyloma* spp. on Compositae and of some specimens from other parts of the world. The European material available has not been sufficient to decide the relationships of certain European and American species, but it serves to indicate the problems involved and the need for critical study of specimens on hand. In the table the specimens are grouped, by hosts, in two categories: first, those assignable to *E. polysporum* or closely related species; and, second, those belonging to *E. Compositarum* or other conidium-bearing species. This arrangement has been adopted in order to emphasize the distinctness of the two phyletic lines involved. The last column in the table gives the preferred name for each specimen; but several of these assignments are open to some doubt, and they should not be accepted without reference to the text where all questionable identifications are discussed under the appropriate host.

Most North American collections fall into one or other of the two well-defined species, *Entyloma Compositarum* Farl. and *E. polysporum* (Pk.) Farl., but there are, on certain hosts, fungi in which some morphological differentiation appears to be constant. As Zundel (21) has noted, *E. polysporum* is very similar to the European species, *E. Calendulae* (Oud.) de Bary, and may have to be included with it. It is kept distinct for the present, but insufficient European material on *Calendula* has been seen to satisfy the writer as to whether the small differences that seem to exist are real and constant. As noted below under *Calendula* and *Erigeron*, the concept of *E. Calendulae* has become seriously confused.





TABLE 1—Continued  
A COMPARISON OF ENTYLOMAS ON VARIOUS COMPOSITAE, GROUPED ACCORDING TO TYPE—Continued

Specimen <sup>1</sup> , name under which issued, and locality	Host	Teliospore dimensions ( $\mu$ )			Spore distribution <sup>2</sup>	Conidia <sup>3</sup>	Preferred name <sup>4</sup>
		Length	Width	Wall			
F. Col. 540	<i>E. arnicale</i>	9.5–15.5	8.5–14.0	1.0–3.0	2	H	<i>E. arnicale</i>
DAOM 6750	"	10.5–17.0	8.5–15.0	0.5–3.0	2	H	"
DAOM 7787	"	10.0–14.5	8.5–14.5	0.7–3.5	2	H	"
DAOM 7788	"	10.0–15.7	9.2–14.5	0.7–3.5	2	H	"
F. Col. 2368	<i>Rumularia arnicalis</i>	9.8–14.8	9.0–12.0	0.7–2.5	2	H	"
H.S.J. (leg. P. Unamono)	<i>E. Calendulae</i>	8.5–14.5	7.0–13.8	0.2–1.5	2	A	<i>E. Compositarum</i>
H.S.J. (leg. P. Cruchet)	"	8.3–13.5	7.4–12.2	0.3–1.2	2	A	"
Sydow Ust. 322 (TRT)	<i>E. Arnicae</i>	8.0–14.2	7.3–12.0	0.2–1.0	2	A	"
F. Col. 5014	<i>E. Compositarum</i>	8.2–13.0	8.0–10.5	0.5–1.5	2	A	"
DAOM 14629	"	8.2–13.5	8.0–13.0	0.5–1.5	2	A	"
H.S.J. (leg. Neger)	"	8.0–13.5	7.2–12.5	0.2–1.5	2	A	"
H.S.J. (leg. P. Cruchet)	<i>E. Bellidiastri</i>	8.5–14.2	7.5–11.3	0.5–1.5	2	A	"
H.S.J. (l. D. & P. Cruchet)	<i>E. Bellidis</i>	8.7–13.0	7.0–11.5	0.3–0.7	2	A	"
S. & E. E. F. C107 (H.S.J.)	<i>E. guaraniticum</i>	8.2–14.5 (15.5)	6.5–11.3	0.2–0.5 (0.8)	2	H	"
E. West	"	9.8–18.3	8.5–15.0	1.0–2.5 (2.8)	4	A	<i>E. guaraniticum</i>
TRT (ex Cornell 15903)	<i>E. Calendulae</i>	8.0–14.0 (15.5)	7.5–12.5	0.2–2.2	2	A	<i>E. Compositarum</i>
Roum. F. Sel. 4869	<i>E. Matricariae</i> f. <i>Chrysanthemi</i>	7.5–15.0	7.2–12.2	0.2–1.0	2	A	"
DAOM 8056	<i>E. Compositarum</i>	8.5–13.5	7.0–11.5	0.4–0.9	2	A	"
DAOM 14646	"	8.5–14.0	7.3–12.0	0.3–0.7	2	A	"
S. & E. E. F. C108 (H.S.J.)	<i>E. Holwayi</i>	8.5–14.2 (15.7)	6.8–13.0	0.7–2.0 (2.5)	2	H	<i>E. Holwayi</i>
DAOM 14459	<i>E. Compositarum</i>	7.7–14.5	7.0–12.8	0.8–2.7	2	A	<i>E. Compositarum</i>
S. & E. E. F. C17 (H.S.J.)	"	7.5–13.5	6.8–11.5	0.2–1.2	1	A	"
DAGM 6867	<i>E. polyporum</i>	8.0–14.0	7.0–12.8	0.3–1.7	2	A	"
DAOM 14365	"	8.5–12.5	7.5–10.3	0.5–1.7	2	A	"
DAOM 14367	"	8.5–13.0	7.0–11.3	0.5–1.4	2	A	"
DAOM 14368	"	8.0–12.5	7.0–10.7	0.7–1.7	2	A	"
DAOM 13837	<i>E. Compositarum</i>	8.5–13.5	7.2–11.0	0.5–1.6	2	A	"
DAOM 14464	"	8.0–12.5	7.3–11.0	0.5–1.4	2	A	"
F. Col. 4820	<i>E. polyporum</i>	8.5–14.0	7.5–13.0	0.7–2.0	2	A	"
F. Col. 4917	"	8.5–14.0	7.3–10.5	1.0–1.7	2	A	"
DAOM 7701	"	8.2–14.0	7.2–11.5	0.4–1.5	2	A	"
DAOM (leg. E. Bartholemew)	<i>E. Compositarum</i>	9.2–12.8	7.2–11.5	0.4–1.5	2	A	"

TABLE 1—Concluded

A COMPARISON OF ENTYLOMAS ON VARIOUS COMPOSITAE, GROUPED ACCORDING TO TYPE—Concluded

Specimen <sup>1</sup> , name under which issued, and locality	Host	Teliospore dimensions ( $\mu$ )			Spore distribution <sup>2</sup>	Conidia <sup>3</sup>	Preferred name <sup>4</sup>
		Length	Width	Wall			
F. Dak. 661 (TRT)	<i>E. Compositarium</i> S.D.	8.5–15.5	7.8–14.5	0.7–2.2	2	—	Doubtful
S. & E. Ec. F. C18 (H.S.J.)	" Ill.	8.5–13.2	7.5–11.0	0.2–0.7	2	A	<i>E. Compositarium</i>
H.C.G. 883	" Wis.	8.5–13.5	7.3–10.5	0.3–1.2	2	A	"
U. of Mich. (ex U. of Wis.)	" Wis.	8.0–14.0	7.3–10.0	0.2–0.7	2	A	"
U. of Mich.	" Mich.	9.2–12.5	7.0–10.0	0.2–0.7	2	A	"
Fl. of Ore. 1826 (H.S.J.)	" Ore.	8.5–12.5	6.8–11.0	0.5–1.4	1	—	"
DAOM (leg. C. J. Johanson)	<i>E. Matricariae</i> Sweden	10.5–16.5	9.2–15.2	0.3–1.5(1.8)	2	A	<i>E. Matricariae</i>
Fl. Hung. Exs. 602	" Hungary	9.7–17.0	9.0–14.5	0.5–1.5	2	A	"
TRT (ex U. of Wis.)	<i>E. Compositarium</i> Wis.	9.2–15.5	7.8–14.0	0.5–2.2	2	H	<i>E. Compositarium</i>
H.S.J.	" Ind.	8.3–14.5	7.8–13.0	0.5–1.4	2	A	"

<sup>1</sup> Abbreviations for specimens, excluding standard exsiccati. DAOM = Mycological Herbarium, Dominion Dept. of Agriculture, Ottawa, Canada. TRT = Univ. of Toronto. U. of Mich. = Univ. of Michigan. U. of Wis. = Univ. of Wisconsin. G.L.Z. = Dr. G. L. Zundel, Pennsylvania State College. H.C.G. = Dr. H. C. Greene, Univ. of Wisconsin. H.S.J. = Prof. H. S. Jackson, Univ. of Toronto.

<sup>2</sup> Under spore distribution, 1 = sparse, 2 = abundant, 3 = crowded, 4 = densely packed.

<sup>3</sup> Under conidia, — = lacking, A = amphigenous, H = hypophyllous.

<sup>4</sup> Some assignments are open to question; the names given in this column should not be accepted without reference to the text under the appropriate host genus.

Emended descriptions of *E. polysporum* and *E. Compositarum* are presented herewith. Information that is readily available in the *North American Flora* is omitted. Information concerning emended exsiccati names will be found below in the text and in Table I.

*Entyloma polysporum* (Pk.) Farl. Teliospores  $9.0\text{--}17.0$  ( $21.5$ )  $\times$   $8.5\text{--}13.5$  ( $16$ ) $\mu$ , densely crowded, displacing most chlorenchyma tissue, wall usually  $1.0\text{--}3.0\mu$ ; conidia and conidiophores completely lacking (Figs. 2, 6).

*Entyloma Compositarum* Farl. Teliospores  $(7.5)$   $8.0\text{--}13.0$  ( $15.5$ )  $\times$   $(6.8)$   $7.2\text{--}11.5$  ( $14.0$ ) $\mu$ , generally abundant, but not densely crowded or displacing chlorenchyma tissue, wall  $(0.2)$   $0.7\text{--}1.5$  ( $2.7$ ) $\mu$ ; conidia generally evident, either falcate  $7.0\text{--}25.0 \times 2.0\text{--}3.5$  ( $5.0$ ) $\mu$ , or acicular  $26.0\text{--}46.0 \times 1.0\text{--}1.5\mu$ ; conidiophores almost always densely crowded in stomata, amphigenous or hypophyllous according to host (Figs. 1, 3, 4, 5).

To avoid undue repetition in dealing with specimens of doubtful identity, all material examined will be discussed under the appropriate host genera. Ciferri species mentioned are described in his 1928 paper unless the contrary is indicated.

**Achillea millefolium.**—*DAOM* 14627 is part of the collection mentioned by Zundel (21) and Bisby *et al.* (2), and is apparently the only record of an *Entyloma* on this host in North America. This specimen and Sydow Ustilagineen 488 are doubtfully distinct from *E. Compositarum*. The Manitoba specimen shows very abundant conidia, but the teliospores are so sparse that the dimensions given probably do not represent the whole range. The lack of conidiophores in the Sydow specimen may be due to the material being old and invaded by saprophytes. *E. Achilleae* Magnus was briefly described as having spores  $12.5 \times 10.5\mu$  and no authentic material has been seen. Liro (10) describes this species as having spores  $19\text{--}18$  ( $9\text{--}18$  intended?)  $\times$   $8\text{--}13\mu$ , and it is possible that two species are involved.

**Ambrosia.**—In addition to the distinctions indicated in the table, *E. polysporum* produces moderately large, round lesions that may be pale, dark green or necrotic, whereas *E. Compositarum* forms small, angular lesions that usually bear masses of conidia on the under surface and may have some above. The issuing of three exsiccati of *E. polysporum* as *E. Compositarum* has doubtless caused much confusion. Fungi Columb. 541 was corrected by Clinton (7), the error of Seym. and Earle Econ. Fungi 292a was pointed out by Savile (13), and Seym. and Earle Econ. Fungi 292b is here corrected for the first time (cf. Figs. 1, 2, 5, 6). It may further be noted that this institution's copy of

FIGS. 1 TO 8. *Photomicrographs of Entyloma spp., from freehand sections in lactophenol with a trace of cotton blue.* FIGS. 1 to 2,  $\times 92$ ; remainder,  $\times 800$ . FIG. 1. *E. Compositarum* in leaf of *Ambrosia elatior*; spores numerous but not crowded. FIG. 2. *E. polysporum* in leaf of *A. elatior*; spores densely crowded. FIG. 3. Spores of *E. Compositarum* in *Gaillardia aristata*. FIG. 4. Conidiophores of *E. Compositarum* massed in stoma of *G. aristata*. FIG. 5. Spores of *E. Compositarum* in *Ambrosia trifida*. FIG. 6. Spores of *E. polysporum* in *A. elatior*. FIG. 7. Spores of *E. polysporum* in *Aster macrophyllus*. FIG. 8. Spores of *E. Davisii* in *Rudbeckia hirta*.





Seym. and Earle Econ. Fungi 294b contains a species of *Stagonospora* associated with the *Entyloma*. This fungus, which has been described elsewhere (Savile, 15), is of interest because of a similar association to be mentioned below under *Lepachys*.

**Arnica.**—The European specimens on *A. montana* are all assignable to *E. Compositarum*. The lesions are small and round. The first specimen listed, though collected in May, was overrun by saprophytes and it is impossible to say whether or not it had possessed conidiophores; it may consist of overwintered basal leaves. The North American collections are all *E. arnicale* E. and E., which is evidently an offshoot of *E. Compositarum* distinguished by slightly larger spores, frequently ornamented with conspicuous curving bands of semicircular cross section. The lesions are moderately large and round. This fungus is definitely not of the 'polysporum' phyletic line. *E. arnicale* is also reported on *A. fulgens* and *A. latifolia*, and it may be noted that the hosts are distributed among the three subgenera of *Arnica* that occur in the area of distribution of the smut, namely British Columbia, Washington, Idaho, Montana, Wyoming, Utah, and Colorado; indeed this area is the distribution centre of the genus *Arnica*, as is clearly shown by Maguire (11).

**Aster.**—*E. Compositarum* was described from *A. puniceus* and probably attacks a considerable number of species of *Aster*. The specimen on *A. VahlII* from Chile shows moderately large, round lesions, but on *A. adscendens* and *A. laevis* the spots are small and angular. The fungus on *A. sericeus*, recently described by Zundel (22) as *E. Aster-sericeanum*, is indistinguishable from *E. polysporum* to which it is here assigned. On *A. sericeus* the lesions are small and variable in shape. The smut on *Aster macrophyllus* is also assigned to *E. polysporum*. If all collections on this host showed the size range of the Wisconsin specimen it might be treated as a distinct species; but the ranges shown by the Ontario specimens are normal for *E. polysporum*, although there is perhaps a preponderance of small spores. The densely packed spores and the complete lack of conidiophores in three abundant collections, all in prime condition, exclude the possibility of this fungus being *E. Compositarum* (cf. Fig. 7). The lesions are strikingly angular and narrowly elongate. In addition to the specimens listed, two in the University of Toronto Herbarium, on *Aster* sp. from Colorado, are typical *E. Compositarum*.

**Bellidiastrum.**—This specimen is typical *E. Compositarum*. The lesions are small and generally round.

**Bellis.**—The specimen is typical *E. Compositarum*. The lesions are small, round, and inconspicuous.

**Bidens.**—Seym. and Earle Econ. Fungi C107 is here assigned to *E. Compositarum*; the maximum spore length is somewhat large, but in other respects the fungus is typical of this species. It is possible that *E. incertum*, published by Ciferri without description but stated to have smaller spores than *E. guaraniticum* Speg., belongs here. The specimen on *B. pilosa*, sent from Florida by Mr. Erdman West, is taken to be the true *E. guaraniticum*. It is unusual in

that it possesses large, densely crowded teliospores, but produces conidia. It is perhaps more closely allied to some South American complex than to either of the main North American phyletic lines. Clinton (7) describes the spores as  $11\text{--}20\mu$  long and more or less adherent in masses or rows. Saccardo (12), in what is presumably an exact translation of the original description, says: "sporis in cellulis parenchymaticis constipatis, globosis sed e mutua pressione saepius irregulariter angulosis,  $10\text{--}14\mu$  diam., crassiuscule tunicatis". This description leaves the full range of spore dimensions in doubt, but in other respects it fits the Florida specimen well except for the failure to mention conidia. However, conidia have so often been overlooked that their absence cannot be assumed unless the author explicitly mentions that they could not be found. Of course, the spores are not actually crowded in the cells; they form intercellularly and their growth causes the collapse and partial disappearance of the parenchyma tissue. The lesions are round in both specimens seen, but are much larger in the Florida specimen than the other. *E. Spegazzinii* Sacc. and Syd. (*E. Bidentis* Speg.) is stated to have even larger spores than *E. guaraniticum*. *E. Bidentis* P. Henn., from Africa, described as having spores  $10\text{--}15 \times 9\text{--}14\mu$  with walls  $1\text{--}1.5\mu$ , might be *E. Compositarum*.

**Calendula.**—The three European specimens examined are presumably typical *E. Calendulae*. The measurements indicate that the spores are slightly smaller than those of *E. polysporum*, particularly as to minimum length and width, and that they are less crowded in the leaf. These are admittedly small differences and adequate study of European material may eventually necessitate *E. polysporum* being treated as a synonym of *E. Calendulae*, as Zundel (21) has suggested. The evaluation of some of the European forms without conidia has proved to be the most difficult part of this study. The New York specimen on *Calendula* is best regarded as *E. Compositarum* although the upper limit of spore length is somewhat high and the spore dimensions agree rather closely with those of the European specimens. It has abundant conidia, whereas all available descriptions and specimens indicate that conidia are never produced on *Calendula* in Europe. The lesions are round in all specimens and vary considerably in size. Unfortunately the concept of *E. Calendulae* has been seriously confused through smuts on various composites being assigned to it with little consideration as to whether or not they were morphologically similar. Later the Sydows (19) broke up the collective species into eight "biological species" without any descriptions, thereby creating seven *nomina nuda*. In so doing they stated that all were morphologically indistinguishable, yet the segregate on *Erigeron* bears conidia and is probably *E. Compositarum*.

**Chrysanthemum and Related Genera.**—There seem to be no records of these plants being attacked in North America, but several species of *Entyloma* have been described from *Chrysanthemum* and *Matricaria* in Europe. *E. Matricariae* Rostr. appears to be a valid species; it somewhat resembles *E. Compositarum* but has decidedly larger spores. The Johanson specimen in the Division Herbarium may be part of the type, which was issued in

Thuemen Mycotheca Universalis 2223. The label of the latter agrees with that on the specimen examined except that the date is given as Sept. 1883, whereas ours is marked 18 Sept. 1883. The specimen can certainly be considered as authentic and makes possible a more adequate description. Conidiophores are plentiful but no conidia were seen. The measurements for Flora Hungarica Exsiccata 602 are combined from the packet in this Division and the one in the University of Toronto; a few conidia were seen and all were about  $16 \times 2.5\mu$  as opposed to  $4-6 \times 2-2.5\mu$  given by Rostrup, a further indication of the variability of conidia. Roumeguere's *E. Matricariae* f. *Chrysanthemi* appears to be *E. Compositarum*. *E. Lagerheimi* Ciferri (*E. Matricariae* Lagerheim) has not been examined; but it is described (Ciferri, 3) as having spores  $20-24 \times 16-18\mu$ , rarely  $28\mu$  diam., and is evidently distinct. Ciferri states in the same paper that *E. Chrysanthemi* Syd., with much larger spores, is *Protomyopsis leucanthemi* Magn. *E. Ludwigianum* was described by Sydow (18) as having spores  $10-13 \times 9-11.5\mu$ . The specimen listed is from the type host and locality and was collected about a month later than the type. It is presumably authentic. As the table shows it is close to *E. polysporum* and may not be distinct. It has small to moderately large, round lesions. D. Sacc. Myc. Ital. 720 on *Chrysanthemum coronarium* (*Pinardia* c.), issued as *E. Calendulae*, is indistinguishable from *E. polysporum*. It produces large, diffuse lesions. This is Ciferri's *E. Scalianum*. Sydow seems to have segregated *E. Ludwigianum* from it mainly because his fungus was collected in Germany and the other in Sicily. The final disposition of these fungi in relation to *E. Calendulae* and *E. polysporum* must await the examination of considerably more material.

**Chrysopsis.**—The two specimens listed, and another from Saskatchewan on *C. hirsutissima*, are typical *E. Compositarum*. The lesions are moderately large and round.

**Cosmos.**—The specimen seen is one of the two exsiccata specimens listed. The type, P. Sydow Ust. 282 from the same locality, has not been examined. The lesions are moderately large and round. Clinton (6, 7) considered this fungus, *E. Holwayi* Syd., to be doubtfully distinct from *E. polysporum*, but in this specimen there are abundant hypophyllous conidiophores. It differs from *E. Compositarum* only in having a few abnormally long spores (Clinton describes them as up to  $17\mu$  long) with rather thick walls; further specimens are needed to show whether these long spores occur regularly. In the meantime it maybe kept distinct.

**Dahlia.**—The three specimens seen all have large, more or less round lesions, and agree very closely except that in the two American collections the teliospores are numerous, whereas in the European fungus they are packed almost as closely as in *E. polysporum*. This is a part of the 'Calendulae-polysporum' complex and may as well be left as *E. Dahliae* Syd. until the whole group is better understood.

**Erigeron.**—A number of North American collections have been made on *Erigeron* spp., especially from the far western United States, and all have



been assigned to *Entyloma Compositarum*. The specimen listed was collected near Ottawa before the large circular lesions had become necrotic, and was assumed to be *Cercospora cana*, which is fairly common locally. It was not studied until some months later; and it has not been rediscovered despite an intensive search. Because no collections on *Erigeron* seem to have been made within some hundreds of miles of Ottawa, it was thought possible that infection might have taken place from nearby *Gaillardia*. A small scale attempt to infect *Erigeron annuus* in 1945 failed, but was inconclusive because hardly any spread occurred even on *Gaillardia*. There seems to be no justification for excluding this fungus from *E. Compositarum*. The identification and naming of the smuts on *Erigeron* have been complicated by the Sydows' (19) treatment of the fungi that had passed as *Entyloma Calendulae*. *Entyloma Erigerontis* Syd. was based upon the fungus on *Erigeron elongatus* (= *E. acris*?) in Norway, and, together with the other undescribed segregates, was stated to be morphologically indistinguishable from *Entyloma Calendulae*. In Ciferri's description of *Entyloma Erigerontis* from Vestergren's Microm. Rar. Sel. 325, which the Sydows apparently had before them when they named the species, the spores are stated to be roundish, 10–15 $\mu$  diam., rarely to 17 $\mu$ , commonly 10–12 $\mu$ , wall 1.0–1.5 $\mu$ , smooth, and conidia are not mentioned; yet Liro (10) describes the fungus as having conidia, and in fact his description fits *Entyloma Compositarum*. The lack of agreement suggests that there might be two species on *Erigeron* in northern Europe. *Entyloma Fischeri* Thuem. was described from Austria on *Stenactis bellidiflora* A. Br. *Stenactis* is a segregate of *Erigeron* and, according to Hegi (9), *S. bellidiflora* is identical with *Erigeron annuus* (L.) Pers., which is well established in parts of Europe. Winter (20) describes *Entyloma Fischeri* as having spores sparse, broadly ellipsoid, translucent yellowish, 14–18  $\times$  12–14 $\mu$ , wall smooth, seemingly thin. Saccardo (12) gives the last figure as 18 $\mu$ , but this is apparently a misprint. The Division material of Roum. Fungi Sel. 4728 on *Stenactis annua* (*Erigeron annuus*) is sterile. This fungus is evidently distinct from *Entyloma Compositarum*. From the published description it is impossible to tell whether it is distinct either from Ciferri's concept of *Entyloma Erigerontis*, or from *Entyloma occultum* Ciferri on *Erigeron spathulatus*, Dominican Republic, with spores 11–21 $\mu$  diam.

**Eupatorium.**—The specimen listed is typical *Entyloma Compositarum*. Neither this specimen nor Ciferri's description support the validity of *Entyloma Eupatorii*.

**Gaillardia.**—It is plain that the fungus on *G. aristata* from Saskatchewan, Manitoba, and eastern Ontario, and on *G. pulchella* from Nebraska, is *E. Compositarum* (cf. Figs. 3 and 4). In view of the findings in other host genera, it is possible that *E. polysporum* does occur on *Gaillardia*, but probably most such reports result directly or indirectly from Fungi Columb. 4820 being issued as *E. polysporum*. Several additional collections from Manitoba and Ontario that add nothing to the dimensions shown in the table have been omitted. The lesions on *Gaillardia* are moderate to large and round. The herbarium

of the Dominion Laboratory of Plant Pathology, Winnipeg, Man., contains no material specifically representing Hanna's (8) demonstration of the formation of conidia in culture by what was thought to be *E. polysporum* from *G. aristata*; but from the evidence presented here it is almost certain that he was dealing with *E. Compositarum*, and the production of conidia was therefore to be expected. *E. Gaillardiae* Speg. (17) has much larger spores and is clearly distinct, if it is actually an *Entyloma*.

**Helenium.**—Both specimens are typical *E. Compositarum* and have large, round lesions. The misnaming of Fungi Columb. 4917 has already been noted (Savile, 13).

**Heterotheca.**—This specimen in the Division Herbarium was collected by E. Bartholemew, Rooks Co., Kansas, 21 Aug. 1895. It was issued, without number, under a Kansas Fungi label, and is *E. Compositarum* as marked. It has small, angular lesions.

**Lepachys.**—The specimens on *L. pinnata* are typical *E. Compositarum*; there is no morphological support for Ciferri's segregation of this fungus as *E. Lepachydis*. It forms small, angular lesions. The identities of the fungi on *L. columnaris* are subject to some doubt. Fungi Dak. 661 has spores slightly larger than is usual for *E. Compositarum*, which probably accounts for Zundel (21) having transferred it to *E. polysporum*; and the walls show some tendency to be banded like those of *E. arnicale*. The spore distribution is as for *E. Compositarum*, but no conidiophores could be found. Conceivably conidiophores might occasionally be lacking from a specimen in good condition in a species where they generally occur; a few specimens have, indeed, been seen in which they were scarce. This specimen is not believed to be *E. polysporum*, but until further specimens have been seen no definite assignments should be made. It forms large, round lesions. When Prof. H. S. Jackson sent this specimen to the writer for examination he noted that another fungus appeared to be present. Examination showed pycnidia of a *Septoria* to be abundant on many of the *Entyloma* lesions and to occur occasionally elsewhere on the leaf. In identifying the *Septoria* as *S. Lepachydis* Fungi Columb. 1875 was examined. This material bore a similar *Septoria*, but it could be seen at a glance that an *Entyloma* was again associated with it. Sections showed that this was yet another fungus. It is of the 'polysporum' type, but, like *E. Davisii* on *Rudbeckia hirta*, the spores have extremely thick walls; it is probably a distinct species, but further material is needed to assess its full range of variation; its lesions are large and round. The *Septoria* seems to be a weak parasite that readily invades tissue attacked by these smuts. Mycologists interested in this complex may find it profitable to examine their specimens of *S. Lepachydis*. The association is evidently similar to that of a *Stagonospora* with *E. Compositarum*, mentioned under *Ambrosia*.

**Madia.**—Although the specimen was collected in the spring (10 May 1914), the leaves were largely dead and are overgrown by saprophytes, which suggests that they had overwintered. Under the circumstances the lack of conidiophores is not surprising and the material may well be left in *E. Compositarum*.

The lesions are small and round. Unless more normal material should reveal distinctions there can be no reason for adopting Ciferri's name *E. Madiæ*.

**Matricaria.**—See under *Chrysanthemum*.

**Rudbeckia.**—The three specimens on *R. hirta*, all issued as *E. polysporum*, are of the same type as that species, but the spores consistently have such remarkably thick walls (Fig. 8) that the adoption of Ciferri's name *E. Davisii* is fully justified. The lesions are generally large and angular. The fungus on *E. laciniata*, segregated by Ciferri as *E. anceps*, is *E. Compositarum*; it forms small, angular lesions.

**Senecio.**—The specimen seen is unquestionably *E. Compositarum*. The type of *E. wisconsinense* Ciferri has not been seen, but the description does not suggest that it is different. The positions of *E. Saccardianum* Scalia and of *E. bavaricum* Syd., which may be identical with it, are doubtful; they may be close to *E. Calendulae*.

It is impossible, without a great deal more material from other parts of the world, to draw definite conclusions about the geographic origins of the morphological types under discussion. The widespread distribution of *E. Compositarum* in North America and the occurrence of *E. arnicale*, a well-marked segregate, in the Rocky Mountains region, suggest that this group originated here and has spread into South America and Europe. Much spread to Europe may have occurred during the last two hundred years along with weed hosts. The comparative restriction of *E. polysporum* to eastern North America and the abundance of *E. Calendulae* and similar forms in Europe suggest that this group originated in the old world; but the morphological distinction of *E. Davisii* indicates that part, at least, of the invasion of the new world took place early. There may be at least one large-spored group that originated in South America, but available descriptions and material are insufficient to give a clear picture. *E. Compositarum* is probably far commoner in South America than the records suggest. Several species erected by Sydow appear to be indistinguishable from it.

There have been very few reports of species of *Entyloma* attacking members of the Cichoreae in North America. The *North American Flora* lists an early report on *Lactuca canadensis* from Minnesota assigned to *E. Compositarum*, *E. bullulum* on *Sonchus oleracea* from Santo Domingo, a fungus on *Agoseris* from Manitoba assigned to *E. Compositarum*, and *E. Agoseridis* on *Agoseris purpurea* from Colorado. The report on *Agoseris* from Manitoba is based on a collection listed by Bisby, Buller, and Dearness (1). Bisby *et al.* (2) later deleted this record, being in doubt about applying the name *E. Compositarum* to a smut on *Agoseris* and the specimen having been mislaid. This collection was made by Mr. I. L. Connors, at Roblin, Man., 28 July 1927. It was set aside with other specimens that required further study, after Mr. Connors was transferred to Ottawa, and was overlooked until recently. The host has been identified with reasonable certainty as *A. glauca*. The smut has teliospores  $8.5\text{--}16.7 \times 7.2\text{--}15.7\mu$ , with walls  $0.5\text{--}2.5\mu$ , abundant but not

crowded; no conidia or conidiophores could be found. It is assumed to be *E. Agoseridis* Zundel, but the type of that species has not been seen. The relationship of this fungus, the one on *Lactuca*, and the various smuts on Cichoreae in Europe is in doubt. It is the writer's belief that all these forms should be held distinct from any attacking members of the Tubuliflorae. The Sydows' breakdown of the collective species *E. Calendulae* was, as has been shown, without regard to morphological distinction. Several specimens on *Arnoseris* and *Hieracium* that have been examined are very similar to *E. Calendulae* (*sensu stricto*). Even if they are removed from *E. Calendulae* they should probably be regarded as one species, but the Sydows' treatment has made almost any action open to misinterpretation. *E. Picridis* Rostr., as exemplified by Sydow Mycotheca Germanica 776 (Univ. of Toronto) has spores  $10.5-17.3 \times 8.5-13.5\mu$ , densely crowded, with walls  $0.5-2.0\mu$ , and lacks conidia. This species was published in 1877 and may prove to be the best depository for certain forms with spores slightly larger than those of the smuts of *Arnoseris* and *Hieracium*.

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## THE AMINO ACID REQUIREMENTS FOR PYOCYANIN PRODUCTION<sup>1</sup>

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AND JACK J. R. CAMPBELL<sup>4</sup>

### Abstract

A medium consisting of acid-hydrolysed casein, glycerol, and a salt mixture has been shown to yield pyocyanin by *Pseudomonas aeruginosa* equal in amount to that obtained from glycerol peptone agar. The monoaminomonocarboxylic acids fraction obtained from casamino acids (Bacto) has been shown to be the source of nitrogen essential to pigment formation. Glycine, *dl*-alanine, *dl*-valine, or *l*-tyrosine have been shown to produce pyocyanin when employed as sole sources of nitrogen. The addition of *l*-leucine to media containing glycine or *dl*-alanine markedly increased pyocyanin formation. The addition of *dl*-phenylalanine to a synthetic medium inhibited pigment production. A synthetic medium consisting of *dl*-alanine or glycine at 0.4% concentration combined with 0.8% *l*-leucine, 1.0% glycerol, and a salt mixture has been shown to be the most suitable medium for pyocyanin production by five representative strains of *Pseudomonas aeruginosa*.

Various synthetic and semisynthetic media have been used in studies of pyocyanin production by *Pseudomonas aeruginosa*. Gessard (2) showed that glycerol peptone agar provided an admirable medium for pyocyanin formation. Jordan (3) reported that the ammonium salts of succinic, lactic, acetic, or citric acids could serve as adequate carbon and nitrogen sources for pyocyanin production. Lourié (4) found that acetate, although suitable for the production of the fluorescent pigment would not support pyocyanin development. Robinson (6) stated that the yield of pyocyanin by *P. aeruginosa* from various synthetic media had never been found to equal that from Bacto peptone. Seleen and Stark (7), in studies on the nutritive requirements of *P. aeruginosa*, were unable to devise a medium more suitable for pyocyanin production than the glycerol peptone agar of Gessard.

The object of the present investigation was to ascertain whether amino acids could replace peptone for the production of pyocyanin and to determine which are essential to pigment formation.

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## Methods

The organism employed in this work was *P. aeruginosa* A.T.C. 9027.

A preliminary study of various media showed that formation of pyocyanin equal to that produced on glycerol peptone agar could be obtained when the following medium was employed:

Casamino acids, 1.0% (Bacto acid-hydrolysed casein)  
Glycerol, 1.0%  
Salt Solutions A and B,\* 0.2 ml. of each per 100 ml. medium  
pH, 7.2

Agar was found not to be essential for maximum pyocyanin formation if the medium was aerated during growth in tubes or if a thin layer of the medium in flasks was employed (10 ml. medium in 125 ml. Erlenmeyer flasks). The latter procedure gave more reproducible results. All cultures were incubated at 30° C. for four days.

For the determination of the amino acids essential to pigment formation, the general technique of Mueller (5) was employed. The casamino acids were separated by the Dakin method (1) into three fractions:

- I. Monoaminomonocarboxylic acids
- II. Proline
- III. Diamino- and dicarboxylic acids

These fractions were employed as the source of nitrogen replacing the casamino acids used in the medium described above.

The extent of pyocyanin production was measured by means of the Fischer Electrophotometer. Ten millilitres of culture were shaken with four successive 2 ml. portions of chloroform. The red acid pyocyanin was separated from chloroform by the addition of 5.0 ml. of 0.1 *N* hydrochloric acid. Five millilitres of 0.1 *N* sodium hydroxide was then added and the resulting blue pyocyanin solution was diluted with an equal volume of distilled water. The density of pigment was measured electrophotometrically. The effect of dilution of pigment on photoelectric readings was found to be a linear relationship.

## Experimental

Fractions I, II, and III, recombined and added to the basal medium of glycerol and salts in the following amounts: Fraction I, 0.5 gm., Fraction II, 0.18 gm., and Fraction III, 0.63 gm. per 100 ml., resulted in pyocyanin formation equal to that obtained when the unfractionated casamino acids were employed as the source of nitrogen.

In order to determine which of the fractions were essential to pyocyanin production, media whose nitrogen sources consisted entirely of individual

\* Salt Solution A: dipotassium hydrogen phosphate, 0.05 gm. per ml.  
potassium dihydrogen phosphate, 0.05 gm. per ml.

Salt Solution B: magnesium sulphate septahydrate, 0.05 gm. per ml.  
potassium chloride, 0.02 gm. per ml.  
ferrous sulphate septahydrate, 0.0002 gm. per ml.

fractions, or of various combinations of the respective fractions at different percentage concentrations, were prepared. Glycerol and Salt Solutions *A* and *B* at the percentages employed in the preliminary study were used throughout. Table I lists the media used and the amount of pyocyanin produced from each medium.

TABLE I

THE EFFECT OF FRACTIONATED CASAMINO ACIDS ON PYOCYANIN FORMATION

Medium*	Fractions of casamino acids	Pyocyanin**
1a	I (0.5%)	29.3
2a	II (0.18%)	0
3a	III (0.63%)	0
4a	I (0.5%) + II (0.18%)	31.5
5a	I (0.5%) + III (0.63%)	68.7
6a	II (0.18%) + III (0.63%)	0
7a	I (0.5%) + II (0.18%) + III (0.63%)	67.2
1b	I (1.0%)	75.5
2b	II (1.0%)	0
3b	III (1.0%)	0
4b	I (0.74%) + II (0.26%)	70.5
5b	I (0.44%) + III (0.56%)	51.7
6b	II (0.22%) + III (0.78%)	0
Casamino acids (1.0%)		61.0

\* Basal medium: glycerol, 1.0%; Salt Solutions *A* and *B*, 0.2 ml. of each per 100 ml. medium.

\*\* Pyocyanin expressed as a reading on logarithmic scale of Fischer Electrophotometer.

It is seen from Table I that when Fraction I is omitted from a medium no pyocyanin formation occurs. When this fraction is present to the extent of 1.0%, even though it is the sole source of nitrogen, pyocyanin production equal to that obtained when unfractionated casamino acids are employed at 1.0% concentration is obtained. At lower concentrations (0.5%) Fraction I employed as the sole source of nitrogen resulted in pyocyanin formation but in smaller amounts. The addition of Fraction II or III to the lower concentrations of Fraction I resulted in formation of increased amounts of pyocyanin. Fractions II and III stimulate the production of pyocyanin in the presence of the lower concentration of Fraction I by providing additional carbon and nitrogen necessary for growth. Thus there is a greater yield of pyocyanin obtained in Medium 4a than in Medium 1a and a still greater increase in Media 5a and 7a. Although in these media the percentage of Fraction I remains constant, there is an increase in the carbon and nitrogen contents parallel with the increase in pyocyanin formed. Medium 1b contains the amino acids essential to maximum pigment formation in addition to sufficient carbon and nitrogen for the growth requirements of the organism.

A medium made up of the nine monoaminomonocarboxylic acids present in Fraction I and combined in the proportions in which they are present in



casein to total 1.0% was next prepared. The composition of this medium, designated as Medium A, is given below:

Medium A*		
Amino acids in Fraction I	Percentage in casein	Equivalent proportion to total 1.0% (1 gm./100 ml.)
Glycine	0.45	0.011
<i>dl</i> -Alanine	1.85	0.046
<i>dl</i> -Valine	6.2	0.155
<i>dl</i> -Isoleucine	6.4	0.16
<i>l</i> -Leucine	9.9	0.248
<i>dl</i> -Phenylalanine	5.2	0.13
<i>l</i> -Tyrosine	5.7	0.14
<i>dl</i> -Serine	0.5	0.016
<i>dl</i> -Threonine	4.2	0.105
	40.5%	1.01 gm.

\* Basal medium: glycerol, 1.0%; Salt Solutions A and B, 0.2 ml. of each per 100 ml. medium.

Nine different media, identical with Medium A except for the omission of one of the respective amino acids constituting Fraction I, were also prepared. A medium consisting of Fraction I at 1.0% concentration served as a control. The amounts of pyocyanin produced from these media are presented in Table II.

TABLE II

THE SUBSTITUTION OF FRACTION I BY THE MONOAMINO-MONOCARBOXYLIC ACIDS OF CASEIN

Medium	Constituents of medium	Pyocyanin
A	Nine amino acids of Fraction I	57.0
1	Medium A minus glycine	38.0
2	" " " alanine	46.7
3	" " " valine	34.7
4	" " " isoleucine	34.5
5	" " " leucine	34.0
6	" " " phenylalanine	67.0
7	" " " tyrosine	54.0
8	" " " serine	51.0
9	" " " threonine	34.0
Control	Fraction I (1.0%)	77.0

The results show that a synthetic mixture of the nine amino acids as a source of nitrogen was satisfactory for pyocyanin production and that more than one amino acid is concerned with pigment formation. The results obtained when phenylalanine was omitted from the medium suggest that the presence of this amino acid may exert an inhibitory effect on pyocyanin production.

In order to study the effect of individual amino acids on the microbial synthesis of pyocyanin, nine media were prepared, employing each of the respective amino acids of Fraction I as the sole source of nitrogen. Except in the case of tyrosine, which was used at 0.6% concentration, the amino acids were made up at 1.0%. The results of this experiment are recorded in Table III.

TABLE III  
PYOCYANIN PRODUCTION FROM INDIVIDUAL AMINO  
ACIDS OF FRACTION I

Medium	Pyocyanin
1. Glycine, 1.0%	25.5
2. <i>dl</i> -Alanine, 1.0%	38.0
3. <i>dl</i> -Valine, 1.0%	29.0
4. <i>l</i> -Leucine, 1.0%	0
5. <i>dl</i> -Isoleucine, 1.0%	0
6. <i>dl</i> -Threonine, 1.0%	0
7. <i>dl</i> -Phenylalanine, 1.0%	0
8. <i>dl</i> -Serine, 1.0%	0
9. <i>l</i> -Tyrosine, 0.6%	18.5

While growth of *P. aeruginosa* was obtained in all media employed, pyocyanin production occurred only in the presence of glycine, alanine, valine, or tyrosine as the source of nitrogen. Alanine gave the highest yield of pyocyanin which, however, did not equal the amount obtained when either casamino acids or combinations of the amino acids were employed. Even when alanine was employed up to a concentration of 2.0% no greater yield of pyocyanin was obtained.

With the object of determining the amino acids essential to maximum production of pyocyanin, media whose nitrogen sources consisted of various combinations of the pyocyanin-producing and non-pigment-forming amino acids of Fraction I were prepared. The media used in this experiment and the results obtained are given in Table IV.

Whereas no pigment is formed when leucine is employed as the sole source of nitrogen, the inclusion of this amino acid in a medium containing glycine, alanine, and valine results in a marked increase in pigment production. Leucine is, however, without influence on the pigment-forming ability of tyrosine. The addition of phenylalanine to a mixture of the pigment-forming amino acids results in the complete suppression of pyocyanin, confirming the observation recorded in Table II.

As the results obtained from these experiments showed clearly that the concentrations of the amino acids used in media were of marked significance with respect to the production of pyocyanin, media made up of various concentrations of alanine and leucine were prepared. Fraction I at 1.0% concentration again served as the control medium. The results are given in Table V.

TABLE IV

THE EFFECT OF VARIOUS COMBINATIONS OF MONOAMINOMONOCARBOXYLIC  
ACIDS ON PYOCYANIN PRODUCTION

	Glycine, %	dl- Alanine, %	dl- Valine, %	l- Tyrosine, %	l- Leucine, %	dl- Isoleucine, %	dl- Threonine, %	dl-Phenyl- alanine, %	dl- Serine, %	Pyocyanin
1	0.2	0.2	0.2	—	—	—	—	—	—	21.0
2	0.4	0.4	0.4	—	—	—	—	—	—	40.0
3	0.2	0.2	0.2	—	0.6	—	—	—	—	87.5
4	0.2	0.2	0.2	—	—	0.6	—	—	—	33.5
5	0.2	0.2	0.2	—	—	—	0.6	—	—	53.0
6	0.2	0.2	0.2	—	—	—	—	0.6	—	0
7	0.2	0.2	0.2	—	—	—	—	—	0.6	24.0
8	—	—	—	0.6	—	—	—	—	—	12.0
9	—	—	—	0.6	0.2	0.2	0.2	—	—	10.5
10	—	—	—	0.6	—	—	—	0.2	0.2	0

TABLE V

THE EFFECT OF VARIOUS COMBINATIONS OF ALANINE AND  
LEUCINE ON PYOCYANIN PRODUCTION

Medium	Alanine, %	Leucine, %	Pyocyanin
1	1.0	—	41.0
2	—	1.0	0
3	1.0	0.2	66.0
4	0.8	0.4	76.0
5	0.6	0.6	68.0
6	0.4	0.8	93.0
7	0.2	1.0	78.0
Control	Fraction I at 1.0% concentration		75.5

TABLE VI

THE STIMULATORY EFFECT OF *l*-LEUCINE ON PYOCYANIN PRODUCTION  
FROM ALANINE, GLYCINE, AND VALINE

Medium	Constituents of medium	Pyocyanin
1	Alanine, 0.4%	35.5
2	Alanine, 1.2%	43.0
3	Alanine, 0.4% + <i>l</i> -leucine, 0.8%	96.0
4	Glycine, 0.4%	18.0
5	Glycine, 1.2%	8.5
6	Glycine, 0.4% + <i>l</i> -leucine, 0.8%	95.0
7	Valine, 0.4%	11.5
8	Valine, 1.2%	27.5
9	Valine, 0.4% + <i>l</i> -leucine, 0.8%	33.0

The maximum yield of pyocyanin was obtained when 0.4% alanine and 0.8% leucine were incorporated in the medium, surpassing pyocyanin production in the control, Fraction I.

A comparative study of the effect of glycine, alanine, and valine, employed as sole sources of nitrogen at two different concentrations and combined with leucine, on pyocyanin formation is presented in Table VI.

The stimulating effect of *l*-leucine on pyocyanin production is shown to be most marked in the case of glycine and alanine and, as a result of these studies, synthetic media for maximum production of pyocyanin by *P. aeruginosa* A.T.C. 9027 have been developed. Either of the media detailed below yielded pyocyanin in quantities greater than those obtained on any other medium.

- |   |   |
|---|---|
| 1. Glycine, 0.4%  | 2. <i>dl</i> -Alanine, 0.4%                                   |
| <i>l</i> -Leucine, 0.8%                                       | <i>l</i> -Leucine, 0.8%                                       |
| Glycerol, 1.0%  | Glycerol, 1.0%  |
| Salt Solutions A and B, 0.2 ml. of<br>each per 100 ml. medium | Salt Solutions A and B, 0.2 ml. of<br>each per 100 ml. medium |

In order to ascertain the general suitability of a synthetic medium for pyocyanin production by *Pseudomonas aeruginosa* it was considered essential to study other representative strains of the species. In Table VII pyocyanin production by five strains on seven different media are compared.

TABLE VII

COMPARISON OF PYOCYANIN PRODUCTION BY DIFFERENT STRAINS OF *P. aeruginosa*

Medium	Constituents of medium	<i>P. aeruginosa</i> A.T.C. 9027	<i>P. aeruginosa</i> 8689	<i>P. pyocyaneus</i> V21	<i>P. aeruginosa</i> R.M.	<i>P. aeruginosa</i> Pa.*
1	Fractions II and III	0	0	0	0	0
2	Glycine, 1.0%	17.0	12.0	21.5	28.0	16.5
3	<i>dl</i> -Alanine, 1.0%	38.0	26.0	30.0	23.0	18.5
4	<i>dl</i> -Valine, 1.0%	29.0	25.0	18.5	20.5	9.5
5	<i>l</i> -Tyrosine, 0.6%	9.5	10.5	7.5	10.0	8.0
6	<i>dl</i> -Alanine, 0.4% <i>l</i> -leucine, 0.8%	97.0	50.0	38.5	50.0	18.0
7	Casamino acids, 1.0%	67.0	31.0	25.5	51.0	20.0

\* We are indebted to Dr. M. Scherago, Department of Bacteriology, University of Kentucky, for culture of *P. aeruginosa* Pa.

The pyocyanin forming ability of the respective strains of *P. aeruginosa* is shown to vary markedly. The synthetic medium in which alanine and leucine are employed as the source of nitrogen is, however, shown to be equal or superior to casamino acids for pigment formation by all strains studied.

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## STUDIES WITH *BACILLUS POLYMYXA*

### V. POTASSIUM AS A FACTOR IN THE PRODUCTION OF 2,3-BUTANEDIOL FROM STARCH<sup>1</sup>

BY H. KATZNELSON<sup>2</sup>

#### Abstract

Potassium either as potassium chloride or dipotassium hydrogen phosphate was found to stimulate production of 2,3-butanediol from starch by *Bacillus polymyxa* and to increase the diol : ethanol ratio. In a casein hydrolysate medium, potassium alone produced this effect; however, in a synthetic (amino acid) medium, phosphorus was found to cause a slight increase in yield of diol especially in the presence of potassium.

Potassium, phosphorus, and magnesium were shown to be required for growth of *B. polymyxa* in a synthetic medium containing glucose, amino acids, and biotin.

By means of 'resting cells' of *B. polymyxa*, acting on glucose, it was demonstrated that potassium specifically stimulated the diol-synthesizing mechanism and that sodium could replace this element partially.

#### Introduction

In common with other living forms bacteria need potassium for growth although the amounts required vary with different species and strains as is indicated by analysis of their ash (14). The role of this element in metabolism has been studied by many investigators. Miller (9), in summarizing the function of potassium in plants, states that it plays a part in the formation of carbohydrates and in the synthesis of protein, that it acts as a catalyst, and that it appears to accelerate enzymatic activity. Further evidence of its role in carbohydrate and nitrogen metabolism in plants is presented by Nightingale (10) and others (4, 15, 16, 17). The influence of potassium on the activity of respiratory enzyme systems has also received considerable attention (2, 8, 11). For example Lasnitzki (8) states that "potassium is able to activate the enzymatic breakdown of glucose with phosphorylation of glucose possibly the initial reaction". This is supported by Boyer *et al.* (2) who demonstrated that potassium acts in the phosphorylation of the adenylic system by accelerating the transfer of phosphate from 3-phosphoglycerate to creatine. Davies (3) noted that potassium is an essential factor in the fermentation of maize by *Clostridium acetobutylicum*, probably being required for growth. In this paper is reported the influence of potassium on the production of 2,3-butanediol from starch.

#### Experimental Methods

The procedure employed and materials used are identical with those reported in the preceding paper of this series (7). Inoculum was prepared by centri-

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Contribution No. 236 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

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fuging a 24-hr. fluid culture, but the cells were washed several times in 0.9% saline prior to final suspension in distilled water in order to reduce carry-over of the elements under investigation to a minimum. Baker's C.P. reagents were used throughout.

### Experimental Results\*

#### Starch Fermentation

In Table I are presented results of omitting the salts included in Solutions A (dipotassium hydrogen phosphate and potassium dihydrogen phosphate) and B (magnesium sulphate, ferrous sulphate, manganese sulphate, and sodium chloride) of Snell and Strong (12) from media containing casein hydrolysate or amino acids. It is clear that the response to inorganic salts is considerably

TABLE I

INFLUENCE OF INORGANIC SALTS ON PRODUCTION OF 2,3-BUTANEDIOL FROM STARCH BY *Bacillus polymyxa*

Strain	Casein hydrolysate medium		Amino acid medium	
	Plus salts	Minus salts	Plus salts	Minus salts
	Diol, %		Diol, %	
39	0.75	0.69	1.10	0.57
47	1.28	0.64	1.38	0.49
82	0.75	0.52	0.98	0.37
252	0.59	0.41	0.74	0.32

greater in the synthetic medium (amino acid) than in the casein hydrolysate medium, which undoubtedly contains various inorganic elements. Strain 47 is most sensitive to salt deficiency even in the casein hydrolysate medium. However, complete suppression of the fermentation was not achieved even with the synthetic medium by omission of these salts owing to the ash content of the starch itself.\*\*

When the individual salts were omitted separately from a medium containing all (with Strain 47 as the test organism) it was found that dipotassium hydrogen phosphate was the only one required for fermentation. Experiments were then carried out with the four strains in an amino acid medium with and without this salt. Analysis† for both butanediol and ethanol were made at three and five days as indicated in Table II. The results show clearly that dipotassium hydrogen phosphate is required by all four strains especially after three days' incubation but that an appreciable yield of diol and ethanol could be obtained after five days in the absence of this salt; this was due without

\* The data presented are representative of the results obtained in repeated experiments.

\*\* According to K. A. Clendenning, Division of Applied Biology, National Research Council, Ottawa, this starch contains 0.26% ash.

† No correction was made for the small amount of acetylmethylcarbinol present in each of the two fractions determined.

TABLE II

EFFECT OF DIPOTASSIUM HYDROGEN PHOSPHATE ON BUTANEDIOL AND ETHYL ALCOHOL PRODUCTION FROM STARCH AFTER THREE AND FIVE DAYS' INCUBATION IN A SYNTHETIC MEDIUM

Strain	Three days						Five days					
	Plus $K_2HPO_4$			Minus $K_2HPO_4$			Plus $K_2HPO_4$			Minus $K_2HPO_4$		
	Diol,	Ethanol,	Diol	Diol,	Ethanol,	Diol	Diol,	Ethanol,	Diol	Diol,	Ethanol,	Diol
	%	%	Ethanol	%	%	Ethanol	%	%	Ethanol	%	%	Ethanol
39	1.09	0.54	2.03	0.57	0.33	1.72	1.25	0.58	2.17	0.92	0.47	1.97
47	1.31	0.60	2.18	0.49	0.28	1.77	1.38	0.58	2.38	1.01	0.47	2.17
82	1.01	0.55	1.85	0.39	0.28	1.39	—	—	—	—	—	—
252	0.71	0.38	1.86	0.30	0.25	1.19	—	—	—	—	—	—

doubt to the slow utilization of the elements in the ash of the starch. The ratio of butanediol to ethanol also shows a marked increase in response to addition of dipotassium hydrogen phosphate after three days' but levels out after five days' incubation. Maximum yields of diol were obtained with 0.2% dipotassium hydrogen phosphate but as little as 0.005% gave almost as high yields.

Since it was still not evident which of the two elements involved, potassium or phosphorus, was more important, or whether both were required, dipotassium hydrogen phosphate was replaced with potassium chloride and disodium hydrogen phosphate and similar results were obtained, as shown in Table III.

TABLE III

INFLUENCE OF POTASSIUM CHLORIDE AND DISODIUM HYDROGEN PHOSPHATE ON FERMENTATION OF STARCH BY *B. polymyxa* (STRAIN 47)

Salts tested*	Casein hydrolysate medium				Amino acid medium			
	Diol, %	Ethanol, %	Diol + ethanol	Diol Ethanol	Diol, %	Ethanol, %	Diol + ethanol	Diol Ethanol
None	0.78	0.36	1.14	2.14	0.48	0.27	0.75	1.79
KCl + $Na_2HPO_4$	1.27	0.53	1.80	2.40	1.24	0.50	1.74	2.45
$Na_2HPO_4$	0.82	0.37	1.19	2.19	0.54	0.30	0.84	1.82
KCl	1.36	0.60	1.96	2.26	1.08	0.50	1.58	2.13

\* 0.1% each.

It appears that potassium chloride alone is capable of satisfying the requirements of this organism in casein hydrolysate medium but not entirely in the synthetic medium. The influence of disodium hydrogen phosphate is negligible in the former but is evident to a slight extent in the latter in the presence of potassium chloride.



As pointed out earlier, the starch used in the above experiments apparently contains sufficient inorganic elements to support some activity of the organism at three days and appreciable activity at five. A very pure starch,\* containing 0.17% ash was then tested in an amino acid medium containing different concentrations and combinations of potassium chloride and disodium hydrogen phosphate as given in Table IV. The data show that addition of potassium

TABLE IV

FERMENTATION OF HIGHLY PURIFIED STARCH AS AFFECTED BY DIFFERENT AMOUNTS OF POTASSIUM AND PHOSPHORUS

Salts tested	Diol, %	Ethanol, %	Diol + ethanol	Diol Ethanol
None	0.16	0.12	0.28	1.29
Complete salt solution (A and B)	0.96	0.43	1.39	2.22
0.005% KCl	0.68	0.36	1.04	1.88
0.01% "	0.81	0.42	1.23	1.93
0.05% "	0.79	0.44	1.23	1.78
0.1% "	1.01	0.45	1.46	2.40
0.1% " + 0.005% Na <sub>2</sub> HPO <sub>4</sub>	0.91	0.41	1.32	2.28
0.1% " + 0.1% "	0.87	0.39	1.26	2.24
0.1% " + 0.2% "	1.02	0.41	1.44	2.45
0.2% " + 0.2% "	1.05	0.47	1.51	2.25
0.1% Na <sub>2</sub> HPO <sub>4</sub>	0.17	0.17	0.34	1.03
0.1% " + 0.005% KCl	0.50	0.26	0.76	1.87
0.1% " + 0.2% "	0.95	0.38	1.33	2.47

chloride alone results in maximum yield of diol under the conditions of the experiment. The fermentation of this pure starch was almost negligible at both three and five days in the absence of the potassium salt. Owing also to its greater purity, this starch was not fermented completely even in the presence of dipotassium hydrogen phosphate; this was accomplished only on the addition of yeast extract.

It cannot be concluded from the above evidence that phosphorus is not required in this fermentation. The organism may obtain enough of this element for its metabolism from the starch but certainly does not obtain sufficient potassium. Earle and Milner (5) found that very pure wheat starch contained 0.13% ash and 0.048% phosphorus, an amount that may satisfy the requirements of the organism.

The influence of potassium on the diol : ethanol ratio is again evident. Added phosphorus exerts no such effect.

#### Growth Studies

The question of the role of potassium in this fermentation naturally arises. Is it required for normal growth of the organism only or does it also specifically catalyse the synthesis of the diol? The influence of various elements on

\* Kindly supplied by K. A. Clendenning, National Research Council, Ottawa.

growth of *B. polymyxa* Strain 47 was studied in a synthetic medium containing biotin (0.3  $\mu$  per litre), glucose 1%, and amino acids. Salt Solutions *A* and *B* were tested first and then their component salts omitted as indicated in Table V. It is obvious that components of both Salt Solutions *A* and *B* are required

TABLE V  
GROWTH OF *B. polymyxa* AS AFFECTED BY DIFFERENT INORGANIC ELEMENTS

Salts added to basal medium	Light transmission, %*
1. None	100
2. Salt Solutions <i>A</i> and <i>B</i>	41
3. 2 minus salts in Solution <i>A</i> ( $K_2HPO_4 + KH_2PO_4$ )	100
4. 2 minus salts in Solution <i>B</i> ( $MgSO_4, MnSO_4, FeSO_4$ )	80
5. 2 minus $MgSO_4$	80
6. 2 minus $FeSO_4$	44
7. 2 minus $MnSO_4$	45
8. $Na_2HPO_4$	75
9. $Na_2HPO_4$ + Salt Solution <i>B</i>	67
10. KCl	98
11. KCl + Salt Solution <i>B</i>	100
12. KCl + $Na_2HPO_4$	81
13. KCl + $Na_2HPO_4$ + Salt Solution <i>B</i>	43
14. KCl + $Na_2HPO_4$ + $MgSO_4$	44

\* Turbidity readings made with Luxtrol colorimeter after 96 hr. incubation; averages of triplicate determinations.

for good growth. These components proved to be salts containing potassium, phosphorus, and magnesium; potassium chloride, disodium hydrogen phosphate, and magnesium sulphate completely substituted for Salt Solutions *A* and *B*. Since addition of magnesium was not required in the fermentation of starch, and phosphorus required only to a limited extent, it may be assumed that these elements were present in sufficient amounts in the starch to permit normal development of the organism. Such was not the case with potassium.

#### Resting Cell Studies

The specific influence of potassium on the synthesis of butanediol was further demonstrated by use of resting cells. These were grown in a medium containing tap water, glucose 1%, peptone 0.5%, and yeast extract 0.05%, dispensed in 100 ml. amounts in Erlenmeyer flasks. After inoculation with two drops of a suspension of cells from a 24 hr. agar slant culture, the flasks were incubated for 20 to 24 hr. at 28° C. The cells from 850 ml. of culture fluid were centrifuged and resuspended twice, brought to a volume of 60 ml. in distilled water, and dispensed in 10 ml. amounts into clean 125 ml. Erlenmeyer flasks containing 1.1 gm. glucose, 0.11 gm. calcium carbonate, and 1 ml. (containing 11 mgm.) of the inorganic salt solutions being tested. Where two salts were used they were combined in the same concentration in 1 ml. solution. The flasks were stoppered and incubated at 28° C., a control flask being kept

in the refrigerator. All flasks were shaken at 15-min. intervals for five hours after which those in the incubator were transferred to the refrigerator over night. Fluctuations in results were very wide unless these conditions were observed. On the following day, the contents of all flasks were transferred to centrifuge tubes, rotated one minute to throw down the calcium carbonate, and then aliquots were removed for determination of 2,3-butanediol and ethanol (7). The diol figures given include acetoin, which was present in small amounts. The ethanol figures were corrected for acetoin that may have distilled over, by a separate determination on the distillate. The averages of seven separate experiments are given in Table VI. Metabolic activity of

TABLE VI

INFLUENCE OF POTASSIUM ON PRODUCTION OF 2,3-BUTANEDIOL AND ETHANOL FROM GLUCOSE BY RESTING CELLS OF *B. polymyxa* (STRAIN 47)

Salts tested	Diol, mM	Ethanol, mM	$\frac{\text{Diol}}{\text{Ethanol}}$	Diol + mM
None	7.16	4.43	1.62	11.59
K <sub>2</sub> HPO <sub>4</sub>	14.56	8.13	1.79	22.69
KCl + Na <sub>2</sub> HPO <sub>4</sub>	13.55	7.45	1.82	21.0
KCl	13.51	7.65	1.77	21.6
Na <sub>2</sub> HPO <sub>4</sub>	9.53	8.32	1.14	17.85

the cells alone was quite extensive as yield of total products indicates. Disodium hydrogen phosphate stimulated increased activity and synthesis of both diol and ethanol but resulted in a sharp drop in the diol : ethanol ratio. Maximum activity occurred only where potassium was present alone or together with phosphorus. The effect of disodium hydrogen phosphate may have been due to phosphorus but may also have been due to sodium, substituting partially for potassium. Use of resting cells provided a means of testing this point. The effect of sodium was not studied in the growth and fermentation experiments since sodium hydroxide was used to adjust the reaction of the medium. Accordingly, potassium chloride, sodium chloride, and disodium hydrogen phosphate were tested by the procedure outlined above, the resulting diol values being, in millimoles: 12.24, 7.00, and 7.88 respectively; the untreated control gave a value of 4.70 mM. Diol : ethanol ratios were identical for the sodium chloride and disodium hydrogen phosphate series and highest with potassium chloride. These data are evidence, therefore, of the partial substitution of potassium by sodium and accounts for the effect of disodium hydrogen phosphate. Added phosphorus exerts little influence, apparently being present in adequate amounts for respiration, within the cells.

Similar experiments with starch were unsuccessful owing to the very small amounts of diol produced under the conditions described.

## Discussion

According to Adams and Stanier (1) the chief products of fermentation of glucose by *B. polymyxa* are 2,3-butanediol, ethanol, carbon dioxide, and hydrogen; in addition, small amounts of acetoin and acetic acid are produced. They assumed too that the normal dissimilatory mechanisms existing in many carbohydrate fermentations are also operative in this fermentation, that is, the carbohydrate breakdown proceeds to a two-carbon compound; butanediol is formed by condensation and ethanol by reduction of this carbon fraction. The work of Stahly and Werkman (13) supports this view in that they found that acetaldehyde, when added to a vigorous culture of *B. polymyxa* caused definite increases in diol, acetylmethylcarbinol, and ethanol. In addition they demonstrated that calcium acetate increased the yield of diol and acetylmethylcarbinol, postulating that acetic acid also plays a part in the biological synthesis of these compounds. It may be first reduced to acetaldehyde, which may condense with itself or pyruvic acid to acetylmethylcarbinol, which is reduced to diol.

It has been shown that potassium caused an increase in yield of both ethanol and butanediol from starch but that the latter accumulated at a faster rate than the former resulting in a higher diol : ethanol ratio. Addition of disodium hydrogen phosphate to the starch medium stimulated little if any activity. However, phosphorus is necessary for growth; consequently it is assumed that the starch contained sufficient phosphorus to permit normal or almost normal growth and fermentation provided potassium was added. Since magnesium is also required for growth it is assumed again that the starch contained sufficient magnesium to satisfy the requirements of the organism.

Attempts to elucidate the effect of potassium were also made using resting cells with glucose as the substrate. Starch did not lend itself to this work under the conditions described. However, the influence of potassium was found to be the same on glucose as on starch, that is, it specifically stimulated diol formation and thus, an increase in the diol : ethanol ratio. These experiments also showed that sodium can substitute partially for potassium, an effect that has been observed by various investigators (6, 9).

Despite the clear-cut evidence of the specific effect of potassium on diol synthesis, however, nothing is known of its actual function in this reaction. Its role as a catalyst or condensing agent in synthetic chemical processes has been recognized by various workers (9). The observed effect on enzyme function may involve a change in the degree of dispersion of the enzyme, in its combining and absorbing power or its degree of dissociation (9). Since potassium is considered "an essential part of the physico-chemical structure of the cell", moving about with considerable freedom, probably in response to acid-base imbalances, it undoubtedly plays a "modifying role in nearly every vital process" (6).

## Acknowledgment

The author wishes to express his appreciation to Miss C. M. Clark for technical assistance.

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## STUDIES ON THE CLADOSPORIUM BLIGHT OF SWEET PEA<sup>1</sup>

By H. M. GOOD<sup>2</sup>

### Abstract

Following the appearance of the *Cladosporium* blight of sweet pea in Ontario in 1945, a study of the causal organism and the disease was carried out. As a result of this study the name *Cladosporium album* Dowson has been retained for the organism in preference to *Erostrotheca multiformis* Martin and Charles, or *Hyalodendron album* (Dows.) Diddens. Temperature and humidity studies have indicated that the parasite and the disease develop most rapidly at 25° C., and that high humidity is necessary for infection and for fruiting on the leaf. Penetration is stomatal, without appressoria, and occurred 10 times as often into leaves with 'open' as into leaves with 'closed' stomata. Evidence that *C. album* may show a positive hydrotropism was obtained. Frequency of penetration increased with an increasing gradient around the stomata, and this was interpreted as evidence that hydrotropism is a factor in penetration. Host range studies and a comparison of frequency of penetration into an immune and a susceptible species showed frequent penetrations into the immune species but no appreciable development in any but sweet pea.

### Introduction

In July of 1945 a blight of the sweet pea, *Lathyrus odoratus* L., caused by *Cladosporium album* Dowson, was found for the first time in Ontario. Although this disease has been reported from a number of widely separated places, no detailed study has been published, and it was felt that its potentialities should be more fully investigated. In the course of the study, the fungus and host were found to be favourable material for an attack on certain general problems of stomatal penetration, and special emphasis has been laid on these.

*Cladosporium album* was first described by Dowson in England in 1924 (6). Subsequently it has been found in the United States, Europe, Western Canada, and East Africa. Losses from the disease have not usually been considered significant, though one report suggests otherwise. In the outbreak that provided source material for this study, the flowers were produced fairly normally, but the injury to the leaves was considerable, and must have caused a significant decrease in yield.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. An abridgment of a thesis presented in May, 1947, to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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## Symptomatology

The conspicuous feature of the disease is a blotching of the leaves with circular to irregular buff areas. These appear first as small yellowish flecks, already becoming necrotic, which spread slowly without forming any definite limiting margin. Single lesions are rarely more than 1 cm. in diameter though several may coalesce to involve most of a leaf. Fig. 1 shows two leaves with lesions of different ages and extents. Similar lesions may occur on the stem.

The length of time taken for the lesions to become macroscopically visible varies greatly with the conditions. In a moist chamber in the greenhouse at a temperature of 25° C., flecks appeared in four to five days, while at 8° C. appearance was delayed for two weeks. High humidity had a slightly accelerating effect on the rate of disease development. Sporulation begins soon after the onset of necrosis of the leaf cells and a powdery layer of white conidiophores and conidia is formed over the buff area of the lesion on both sides of the leaf. Some conidiophores may arise from the green tissues surrounding the necrotic region, but these are never numerous. Often there is little sporulation, particularly on plants grown under winter conditions in the greenhouse. On these, small necrotic areas with few conidiophores were all that appeared when the plants were held several weeks after inoculation.

The development of the disease is more limited in seedling leaves than in adult, partly because most infected seedlings drop their leaves within 10 days or two weeks after inoculation. Dropped leaves showed almost no sporulation, even when placed in a moist chamber immediately after falling. Fruiting of *C. album* was light under greenhouse conditions in Toronto.

Both spring-flowering and summer-flowering types of sweet pea were attacked in the source outbreak. The spring-flowering ones appeared more seriously damaged but since they were well past their best season when first seen, little significance can be attached to this appearance. In greenhouse tests on seedling plants no difference in reaction was evident.

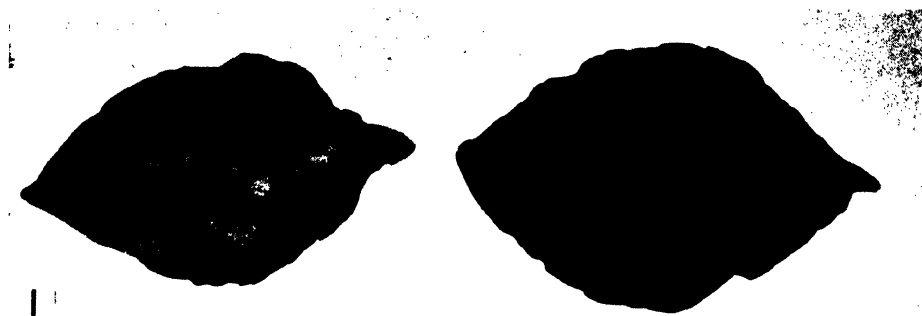
This picture of the symptoms agrees closely with Dowson's observations (6) except for the poor sporulation, and the dropping of seedling leaves before the lesions had much more than appeared. Dowson found good sporulation on most of the infected leaves, and defoliation only with leaves with large necrotic areas. All the work reported here has been done under glass in Toronto where conditions for growing sweet peas are not ideal. The plants have been significantly less robust than those pictured in Dowson's report and it is thought that this is sufficient to explain any differences noted. A tendency for spindling plants, grown in winter under very low light intensity, to show least sporulation and earliest defoliation, supports this view.

FIG. 1. Leaves of sweet pea showing lesions caused by *Cladosporium album*.

FIG. 2. Microconidia of *C. album* on submerged mycelium from a cornmeal agar slant.  $\times 1000$

FIG. 3. Types of cultures of *C. album*. From left to right strains (a), (b), (c), and (a).

FIG. 4. Microsclerotium of *C. album* submerged in cornmeal agar.  $\times 750$







## Isolation and Study in Pure Culture

The fungus was isolated directly from the diseased leaves using a dry needle. In most cases this resulted in a pure culture uncontaminated by bacteria. Some single-spore culturing was done but the bulk of the transfers were of mass material.

Study of the fungus on a wide range of media showed that the type of colony varied considerably depending on the substrate. On cornmeal agar, a powdery layer of conidiophores, with little suggestion of felting, was obtained. This type of colony also formed on Czapek's and Barnes's agars. However, on potato dextrose, malt, prune, pea, and nutrient agars, a raised and felted colony developed with large quantities of sterile mycelium. The colonies rarely became more than 2 cm. across. From the agars tested, cornmeal was finally adopted for most of the studies since it gave a considerable yield of spores and a type of colony with little sterile mycelium. This was an advantage in studying the morphology of the fungus. Moreover, the work of Martin and Charles on *Eurostrothea multiformis* (18) was done on cornmeal and it was desirable to use the same medium as these authors. In cases where the extent of growth was to be measured, either prune or potato dextrose agar was used.

The influence of temperature on the development of the fungus in culture was studied with reference to both the growth of colonies for a number of days and the growth of germ tubes for a 48 hr. period. The fungus developed normally within the range of 5° C. to 30° C. Some development took place at temperatures lower than 5° C., but growth was extremely slow. At temperatures higher than 30° C., germination occurred, but the fungus was not able to continue beyond this stage. The optimum temperature lay in the vicinity of 25° C.

*Cladosporium album* was found to be tolerant to considerable range in pH. Using both colony development and germ tube growth as criteria, good growth was obtained within the range of pH 4 to pH 8 with a maximum rate at pH 6.

The effect of relative humidity on growth and sporulation in culture was studied using a method adapted from Hopp (15). A stream of air was passed through a saturated solution of a salt to give a definite relative humidity, and then was directed onto the surface of an agar slant on which spores of *C. album* had been placed. Humidities of 45, 65, 80, 90, and 100% were obtained using calcium chloride, sodium nitrite, ammonium chloride, sodium carbonate, and water. In order to maintain the substrate at a constant hydration, the tubes containing the slants were pierced with two holes and were placed in a water-bath so that water lost from the upper surface could be replaced from below. Under these conditions, relative humidity had no effect on the type of growth, or time or extent, of sporulation. This was interpreted as evidence that the fungus is capable of transporting water along the conidiophores and spore chains rapidly enough to support growth at all humidities tested, provided the supply of water to the absorbing organs is adequate. Further

mention of these results will be made in connection with the influence of relative humidity on disease development.

A tendency for *C. album* to be somewhat unstable in culture was noted during the investigation. No aberrant types of cultures were seen for the first few months, but at the end of six months a number of different types had appeared. These were purified by transfer and by single spore isolation and ran true to type in all cultures but one. In this manner three distinct types, illustrated in Fig. 3, were obtained: (a) powdery white type slowly forming dark mycelium in the substrate; (b) powdery white type forming dark mycelium and microsclerotia in a few weeks; and (c) fluffy white type slow to form dark mycelium, which usually forms in masses rather than in microsclerotia. This last type was sterile and resembled a culture obtained from the Centraalbureau voor Schimmelcultures derived from a collection of Dowson's. In infection experiments there was no difference in pathogenicity between these various forms except that the sterile forms did not fruit normally nor did they form substomatal knots.

### Morphology and Taxonomy

Various reports of the white blight of sweet pea have dealt differently with the taxonomy of the causal organism. When first describing the fungus in 1924, Dowson (6) decided that it should be considered as a *Cladosporium* though it was entirely hyaline. In 1928, Martin and Charles (18) accepted the name *C. album* and reported that they had obtained the perfect stage for which they created the new genus and species, *Erostrotheca multiformis*. This ascomycete was said to form a number of types of spore in culture, viz. *Ilormodendron*, *Ovularia*, *Ilaplaria*, *Pseudofumago*, and *Pseudosaccharomyces*. In 1934, Diddens (5) transferred *C. album* to the genus *Ilyalodendron*, which he had founded upon wood-pulp fungi from Sweden. In view of these reports, special attention was given to the morphology and taxonomy of the fungus.

The principal type of spore found was the *Cladosporium*. These spores were borne in branched chains (Fig. 7) and were mostly one-celled and oval though a few long, cylindrical, two-celled ones occurred as basal elements of the chains. The *Cladosporium* spores ranged in length from 3 to 16  $\mu$  and in width from 3 to 5  $\mu$ .

A second type of spore was also found though not with regularity. These were small (1 — 1.5  $\times$  2 — 5  $\mu$ ) and were borne singly or in agglutinated groups, usually on branched conidiophores (Figs. 2 and 6). They occurred submerged in the medium and only in cultures approximately one month old, apparently always associated with dark microsclerotia (Figs. 4 and 5) typical of the 'b' strain described above. The other strains showed neither microconidia nor distinct microsclerotia though masses of dark mycelium sometimes formed.

These microconidia and microsclerotia suggest spermatia and perithecial initials of an ascomycete. However, no signs of the perfect stage appeared even when mixtures of all the available cultures were made. It is possible

that *C. album* is an ascomycete that is undergoing loss of functional sexuality or that it is heterothallic and only one strain was available. Unfortunately no decision could be reached on this point. Attempts to get cultures from various parts of the world, where *C. album* occurs fairly regularly, have so far been unsuccessful.

No further noteworthy structures were found in the Ontario material of *C. album* though a suggestion of chlamydospore formation was observed in some of the older cultures and some germinations resembling budding were seen. These suggest the *Pseudofumago* and *Pseudosaccharomyces* forms of

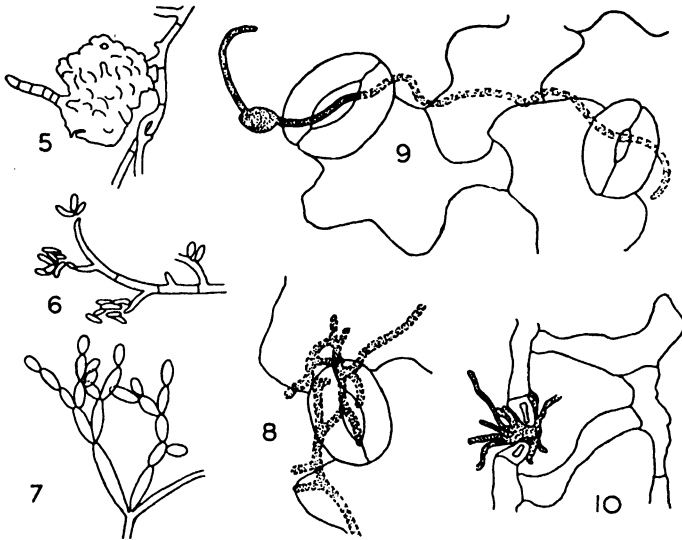


FIG. 5. *Microsclerotium* of *C. album*.  $\times 370$

FIG. 6. *Microconidia* of *C. album*.  $\times 580$

FIG. 7. *Macroconidia* of *C. album*.  $\times 370$

FIG. 8. *Initiation of a substomatal knot just prior to fruiting*.  $\times 580$

FIG. 9. *Penetration of C. album through stoma of sweet pea*.  $\times 580$

FIG. 10. *C. album fruiting on leaf of sweet pea*.  $\times 350$

*E. multiformis*. However, since Martin and Charles state that in certain of these forms the transitions can be easily understood and interpreted from a single slide and no such distinct combinations were found in the Ontario collections of *C. album*, it is thought that these occasional occurrences cannot represent the *Pseudofumago* and *Pseudosaccharomyces* of *E. multiformis*.

A comparison of the observations on the Ontario collection of *C. album* with those of Martin and Charles on *E. multiformis* shows that while a number of similarities exist, the range of imperfect spore forms can only be made to agree by considering certain unusual growth forms as spore types. The *Hormodendron* type exists in the Ontario material but not as a form distinct from the *Cladosporium*. The microconidia resemble the *Ovularia* and *Haplaria* stages of Martin and Charles but there are not two distinct types. They

agree more closely with the description of *Haplaria* though they did not develop nearly so quickly, as was reported for *Erostrothea*. No regular occurrences of the *Pseudofumago* and *Pseudosaccharomyces* stages were found and neither of these stages was sufficiently clearly marked to be considered as a spore type.

In addition to what are considered as differences in the spore forms occurring in the Ontario collection of *C. album* and those found in cultures of *E. multiformis*, one other consideration should be made regarding the connection of *C. album* and *E. multiformis*. In Martin and Charles' paper, the cultures of *E. multiformis* are shown as widespread and zonate, approximately covering a Petri dish. In both general appearance and extent of growth, these differ radically from those found characteristic of any strain of the Ontario collection and from those described by Dowson. Considering these differences and the lack of definite agreement in the morphology in culture, it is felt that there is some doubt that *E. multiformis* is the perfect stage of *C. album*.

Diddens' removal of *C. album* from the genus *Cladosporium* brings up the question of the proper name for the imperfect form. Apart from colour, the sweet pea fungus shows close resemblance to other Cladosporia and the question is whether colour alone is a sufficient basis for excluding the fungus from that genus. Dowson gave considerable attention to this problem and decided that the fungus could be properly called a *Cladosporium*, the close resemblance to *C. herbarum* was noted by Brooks (6) and in parallel cultures with *C. fulvum*, no difference could be made out until after the onset of fruiting in both forms. This and the fact that *C. fulvum* mutates to a white form indicates that colour is of little fundamental importance in determining taxonomic relationships. While it is realized that the present classification of the Imperfects is an artificial one, based to a large extent on such factors as colour, it is felt that in the case of the sweet pea fungus, the name *C. album* is more indicative of the type of fungus concerned than is *Hyalodendron album* and probably indicates as much actual relationship. The name *C. album* Dowson has therefore been retained.

## The Influence of Environment on Disease Development

### Temperature

A series of air temperatures was obtained by covering the soil cans of Wisconsin temperature tanks with perforated cellophane. The temperature of the air in the cans agreed closely with that of the water in the tanks. Plants used in these experiments were removed from the soil and their roots placed in normal Pfeffer's solution to avoid the high carbon dioxide production of the soil in the rather poorly ventilated chambers. The leaves were inoculated by brushing a suspension of spores over the surface and the plants were placed in cans with moist paper towels around the sides to give high humidities. Leaves were removed, cleared, and examined at the ends of two, four, and five days,

and later checks were made at 10 days and three weeks at the lower temperatures. The examinations provided a picture of the penetration and development at various temperatures.

At 35° C. a few spores had germinated at the end of two days. However, no further growth took place and the leaves died in a few days. These observations agree with those on temperature effects in culture in indicating that the fungus is unable to develop appreciably at 35° C.

At 30° C. germination was good after 48 hr., but the germ tubes were short and no penetrations were seen. After four days the amount of growth had increased but there were still no penetrations. Distinct indication of the disease did not appear till 10 days after inoculation.

At 25° C. growth was extensive after two days, and penetrations were noted. Macroscopic symptoms of the disease appeared in five days. A slightly slower development took place at 20°, but very minute flecks showed on the fifth day.

At 15° C. most of the spores had germinated after two days, but the germ tubes were short. However, within four days penetration had occurred, and macroscopic symptoms appeared in six days. At 8° signs of germination appeared in two days, but it was three weeks before definite flecks appeared.

The disease was found to develop within the range of 8° to 30° with an optimum at 25° C. The close parallel between the effect of temperature on the development of the disease and on the growth of the parasite suggests that in influencing the rate of development of the disease, temperature is acting principally upon the parasite.

### *Humidity*

On glass slides and on leaves in air chambers with good circulation, spores of *C. album* did not germinate at relative humidities lower than 95%, but on leaves in stagnant air, good germination was obtained at relative humidity 90%. This is apparently due to the modifying influence of the leaf on the air at its surface (Yarwood and Hazen (24) ). A significant amount of water must be lost through the stomata even at humidities as high as 90%.

The influence of humidity on postinfection development was studied by placing plants in bell jars into which air was passed via towers of saturated salt solution to give definite relative humidities. Plants were inoculated and held in saturated air for 48 hr., then placed in the controlled humidities.

It was found that the leaves in air at 100% and 90% developed fruiting lesions in eight days, though the conidiophores were noticeably shorter at 90%. Lesions were clearly marked at all the lower humidities by the 11th day, but no fruiting developed. Apparently development in the leaf is slowed down only slightly by low humidities, but sporulation is prevented below 80% of saturation.

The studies on growth of *C. album* in culture showed that conidia may be produced abundantly in dry air if the substrate is at maximum hydration. The discrepancy in the reaction of *C. album* to low humidity in culture and on

the leaf suggests that the effect of relative humidity on fruiting on the leaf may be primarily an effect on the hydration of the collapsed leaf tissues in which the fungus grows, and that under dry conditions there is not sufficient moisture available for the production of conidiophores.

### *Host-Parasite Relations*

Contrary to Dowson's preliminary report (6), the fungus has been found to penetrate through the stomata (Fig. 9). No exceptions to this rule have been seen in an examination of thousands of cases. Penetration may occur almost as soon as the spore germinates or after the germ tube has grown several millimetres over the surface of the leaf. In many cases a side branch penetrates. No appressoria have been observed.

There is often an increase of the germ tube diameter immediately after the stomatal aperture is passed. This increase is usually only of the order of 20% but thin hyphae may double their diameter. After penetration, the hypha turns sideways and runs subepidermally, usually for the width of two or three epidermal cells though in some cases much farther. The spread through the leaf is by these straight, infrequently branched, runner hyphae. The host cells do not at first show any reaction and signs of collapse of the leaf tissues appear half a dozen or more cells behind the advancing hyphal tip. As the runner hyphae become branched through the mesophyll, the host cells show first a slight shrinking, then complete collapse. In the collapsed cells, the chloroplasts are no longer distinct though the cell retains a suggestion of green colour for a short time. Later the necrotic areas turn brown. The infected part of the leaf collapses following death of the cells and is much thinner than the healthy portion. Fruiting usually occurs from the necrotic area in which the fungus has spread through both spongy and palisade mesophyll. A hyphal knot forms in the substomatal chamber (Fig. 8) from which conidiophores grow out through the stomata (Fig. 10) and bear branched chains of conidia.

### **Penetration Studies**

#### *Materials and Methods*

The studies on penetration phenomena have consisted essentially of a comparison of the effects of various conditions on the frequency of penetration. The method of estimating frequency described here remained the same throughout all experiments on the leaf. Most of the series were done on cut stems. It is perhaps desirable to use the whole plant but the branches appeared perfectly normal for the duration of the experiments, and the control methods used could not readily be applied to potted plants.

Leaves were inoculated with a water suspension of spores brushed on the under surface with a camel's hair brush and spread by gentle rubbing with the fingers until an even film was obtained. The leaves were allowed to dry and were then placed in the required conditions. At the end of the time allowed, the leaves were removed, boiled in alcohol to remove the air and chlorophyll, and cleared in lactophenol. When a leaf was to be examined it was mounted

in lactophenol cotton blue. This gave a very clear leaf in which all layers could be made out easily. The spores and mycelium on the leaf stained dark blue, while that in the leaf remained unstained. A large number of microscope fields were then examined and the number of penetrations noted. The same number of fields was examined for each leaf of any one series and the same number of leaves for each environment. The fields were chosen at random along a path that zigzagged back and forth across the leaf in such a way that fields from all parts of the leaf were examined.

Since the areas examined were equal, the number of penetrations counted was taken as a measure of the frequency of penetration. Repeated counts on the same leaf have shown that this method of estimating frequency is accurate to within a few per cent where appreciable numbers of penetrations occur. The variation from leaf to leaf of the same series is considerably greater, but results have been consistent and for the most part have been highly significant statistically. Analysis of the data from one series on the influence of relative humidity on the number of penetrations gave a mean for six leaflets in saturated air of  $12.6 \pm 1.5$ , while in the same series the mean for six leaflets at a relative humidity of 96% was  $39.1 \pm 5.8$ . The difference of these means is five times the standard error of the difference, giving a significance beyond the 1% level. The variation of the individual counts and the mean in the above experiment is typical of the results obtained. Actual counts have therefore been recorded in summary only.

#### *The Influence of Stomatal Aperture on Frequency of Penetration*

The influence of stomatal aperture on frequency of penetration was determined by estimating the numbers of penetrations into leaves in which the stomata were either 'open' or 'closed'. The condition of the stomata was determined by making a stripping into absolute alcohol by the method of Loftfield (17), and the numbers of penetration determined as described above. When almost all of the stomata showed no aperture they were classed as closed and when most showed a considerable opening they were classed as open.

It was found that the stomata did not follow the light and dark experience of the plants but tended to show some opening during the night. In moist chambers, this tendency was more pronounced and some remained open for several days in complete darkness. The period of darkness necessary to obtain closure varied with the previous light experience of the plants, presumably due to the resultant changes in reserve food. The stomata could also be induced to close by causing branches to wilt slightly, and some success was had in controlling the aperture of the stomata in epidermal strippings floated on sucrose solutions of various osmotic pressures. By using these various methods of controlling aperture, counts were obtained of the number of penetrations into leaves with closed and open stomata. The results of the experiments conducted are summarized in Table I.

In Experiments 1, 2, and 3, plants were placed in moist chambers in the greenhouse and in the darkroom. After two days the stomata in the dark



TABLE I  
THE FREQUENCY OF PENETRATION OF *C. album* INTO LEAVES  
WITH 'CLOSED' AND 'OPEN' STOMATA

Experiment No.	Total leaflets examined	Total fields examined	Penetrations 'open stomata'	Penetrations 'closed stomata'
1	16	3200	93	7
2	20	4000	30	3
3	24	2400	196	18
4	22	2200	22	5
5	28	3000	61	15
6	28	2800	300	14
7	—	264	27	6
8	—	660	23	1
9	—	200	17	4
Totals	138	18,724	769	73

were closed. The plants were inoculated and left in the moist chambers for four days more after which the leaves were fixed and counts made. Checks on the stomatal apertures were made occasionally throughout the period in the moist chamber. The stomata in the dark were almost all completely closed while most of those in the greenhouse remained open. It should be stressed here that neither in this experiment nor in any of the others reported was complete closure nor complete opening of all stomata observed. The counts showed that 28 penetrations occurred in the dark and 319 in the light.

In Experiment 4, leaves were treated as in the above case except that one lot of leaves was prestarved in the dark prior to inoculation. Both lots were inoculated and placed in dark, moist chambers. The prestarved plants showed closed stomata throughout the infection period, while the others remained open for 48 hr. after inoculation. At the end of that period they were fixed and examined. Five penetrations occurred in the leaves with closed and 22 in those with open stomata. Unfortunately this experiment could not be repeated within the season of light intensity that was essential to the stomata of unstarved plants remaining open for any considerable period in the dark.

In Experiments 5 and 6, leaves on cut stems were inoculated and placed in moist chambers in the greenhouse over sulphuric acid to give a relative humidity of 95%. Half of the stems were placed in a flask with water and half in an empty flask. Counts made on the third day after inoculation showed that 29 penetrations had occurred into the leaves with closed stomata, and 361 into those with open.

Experiments 7, 8, and 9 were carried out on strippings on sucrose solutions. Leaves were inoculated and placed in moist chambers until some germination had occurred. They were then stripped and floated on solutions in covered watch glasses. After two days the strippings were mounted in lactophenol cotton blue. Eleven penetrations occurred through closed and 67 through open stomata.

Throughout the nine series, 73 penetrations were observed into the leaves with closed and 769 into those with open stomata. Results with all methods were consistent in indicating many more penetrations where the stomata were open. It must be admitted that at least one factor varies in all the above experiments, but it is felt that the number of methods used and the consistency of the results answers any criticism based on these differences. We may conclude that penetration of sweet pea leaves by *C. album* is greatly impeded by closure of the stomata.

It is interesting to compare these results obtained with *C. album* with those of other workers. Hart (13) evolved a theory of "functional resistance" of wheat to stem rust based on the inability of the rust to penetrate closed stomata, but this "functional resistance" was questioned by Peterson (21). Later, Caldwell and Stone (3) showed that germ tubes of *Puccinia triticina* Eriks. penetrated closed stomata as frequently as open. Hart and Forbes (14) investigated a number of rusts and found that light experience at the time of infection influenced the amount of infection in some cases but did not in others. Pool and McKay (22) reported that germ tubes of *Cercospora beticola* Sacc. did not penetrate closed stomata of the sugar beet.

With the exception of Pool and McKay (22) all the reports mentioned above deal with the rusts. Even in that group the behaviour does not appear to be consistent, though it seems that at least some rusts penetrate closed stomata. Possibly they are able to do this by virtue of the appressorium, which usually forms over the stoma. So far as is known, penetration through closed stomata has not been shown for fungi that do not form appressoria such as *C. album* and *Cercospora beticola*. This suggests that exclusion may be mechanical, though evidence will be presented in the following section that a type of exclusion may result from suppression of factors that guide the germ tubes to the stomata. It may be that there is a fundamental difference in the mechanisms of penetration with and without appressoria, but further comparative work is necessary before any conclusion can be reached on this point.

### *The Role of Hydrotropism in Penetration*

#### *Hydrotropism on an Artificial Membrane*

It has been suggested a number of times (Bond (2), Balls (1) ) that germ tubes of fungi that normally penetrate the host through stomata are attracted to these by the water vapour transpired. As a preliminary test of this hypothesis an experiment was designed to see whether *C. album* showed any hydrotropism under controlled conditions that simulated those of the leaf epidermis. The data obtained in the experiments on *C. album* have been augmented by several series on *C. fulvum*.

Experiments were set up in the following manner. Van Tieghem cells were cemented to microscope slides, and the upper rim covered with a layer of paraffin. The cell was half-filled with water, and a piece of very thin aluminium foil was sealed over the cell by being pressed down around the edges with

a warm spatula. The foil was pierced with small holes about  $50\mu$  in diameter and 2 mm. apart using a fine glass needle and spores from a young colony of *C. album* or *C. fulvum* dusted over the surface of the membrane. This was done by lifting the surface of an agar slant culture with a long spatula. The agar stuck to the spatula sufficiently that the culture could be inverted over the membrane and pressed lightly on it, dislodging a large number of spores. The slides were then placed in moist chambers in which the humidity was controlled. In most series a humidity of 95% was obtained with a sucrose solution, or 100% with pure water. The moist chambers were placed in a constant temperature oven at 25° C.

Observations were made on the slides after two or three days depending upon the rapidity with which the spores germinated. The method of studying the effect of the conditions imposed was adapted from that of Graves (9) in which the directions of large numbers of germ tubes growing adjacent to the holes was analysed to show whether or not there was any directive force. Only spores lying one diameter of the hole or less from its edge were considered. Imaginary crossed lines giving quadrants, *A*, *B*, *C*, and *D*, were placed upon each spore and the germ tubes classified according to the quarter into which they grew. A preponderance of *A* types would be expected if there were an attraction towards the hole, a preponderance of *C*, if there were a repulsion, and equal numbers of all if there were no effect.

The results of the experiments are given in Table II. In all cases in which there was a gradient of increasing moisture as the holes were neared, there was a considerably larger number of germ tubes growing towards the hole than away from it. In the control experiments where there was no moisture gradient, there was no significant difference in the numbers in the various classes.

TABLE II

DIRECTION OF GROWTH OF GERM TUBES ON A PERFORATED ALUMINIUM MEMBRANE SEPARATING AREAS OF DIFFERENT RELATIVE HUMIDITIES. CLASS *A* INCLUDES THE GERM TUBES GROWING TOWARDS THE HOLE, CLASS *C*, THOSE AWAY FROM THE HOLE, AND CLASSES *B* AND *D*, Laterally

<i>R.H.</i> (ext.)	<i>R.H.</i> (int.)	Fungus	Class <i>A</i>	Class <i>B</i>	Class <i>C</i>	Class <i>D</i>
95	100	<i>C. album</i>	48	20	15	24
95	100	"	38	9	6	9
95	100	"	24	7	1	11
95	100	<i>C. fulvum</i>	21	2	0	1
95	100	"	31	1	2	2
95	100	"	113	27	14	26
95	100	"	173	56	53	59
95	100	"	448	194	105	201
100	100	<i>C. album</i>	8	7	7	7
100	100	<i>C. fulvum</i>	86	79	87	74
100	100	"	45	41	47	39
100	100	"	96	91	89	90

The behaviour of germinating spores on an aluminium membrane under the conditions imposed shows that there is a hydrotropic response in both *C. album* and *C. fulvum* to a gradient of the magnitude likely to be found on a leaf during the inoculation period. This demonstration of the hydrotropic response of *C. album* and *C. fulvum* is one of the few dealing with the response of fungi to volatile substances. Balls (1) noted that germ tubes of *Puccinia glumarum* var. *Hordei* Eriks. grew from room air through perforations in a rubber membrane towards a saturated atmosphere but showed no tendency to return. Miyoshi (19), Fulton (7), and Graves (9) have dealt with chemotropism of fungi in, or on, a liquid or agar substrate, but their results have little or no bearing on problems of stomatal penetration. It is hoped that the methods described here may prove applicable to a study of the influence of volatile substances other than water on the direction of growth of germ tubes.

#### *Hydrotropism on the Leaf*

If it be true that germ tubes are attracted to stomata when the air at their mouths is more humid than in the regions of the germ tube, then the number of penetrations should be increased by an increase in the circumstomatal gradient. This increase may be achieved by decreasing the relative humidity of the air around the leaf. The influence of the gradient on the frequency of penetration should then be shown by an increase in the number of penetrations with decreasing humidity. Humidity values given are those at the surface of the control solutions approximately one-half inch away from the leaf.

It was not found possible to make a direct measurement of the effect of relative humidity on the frequency of penetration. First experiments in which inoculated leaves were incubated in moist chambers over sucrose solution to give relative humidities of 90 to 100% (Shaw (23) ) showed that the number of penetrations tended to increase with a decrease from 100% to 97% of saturation and then decreased till there were very few at relative humidity 90%. There was evidence in these experiments that the sharp reduction in growth in the lower 90's was responsible for the decrease in number of penetrations.

Since it appeared that the real influence of humidity on frequency of penetration could only be determined after eliminating the variations due to different extents of growth, an investigation of the relation between number of penetrations and length of germ tubes was carried out. Leaves were inoculated, placed in control chamber at relative humidity 100%, and then in constant temperature growth chambers at 22.5° C. and 15° C. (12° in one experiment). At intervals of approximately 12 hr., samples of four leaflets were removed and the frequency of penetration and average length of germ tubes estimated. Results of three such pairs of experiments are given in Table III. Judging by the correlation coefficients, there is a close approximation to a linear relation between length of germ tube and frequency of penetration. This provides us with a means of obtaining comparable frequencies even when the extent of growth is not equal.

TABLE III

RELATION OF AVERAGE LENGTH OF GERM TUBES IN  $\mu$  TO NUMBERS OF PENETRATIONS

Series No.	Temperature, ° C.	Paired readings						Correlation coefficient	Regression coefficient
1	22.5	Length	0	62	122	162	205	.99**	2.4 $\pm$ .2
		Penetration	0	20	38	67	94		
	15	Length	0	37	91	178		.91*	2.1 $\pm$ .6
		Penetration	0	10	34	68			
2	22.5	Length	0	56	125	105	195	.98**	5.3 $\pm$ .7
		Penetration	0	51	109	190	216		
	15	Length	0	86	101	151	183	.98**	5.3 $\pm$ .6
		Penetration	0	85	121	155	175		
3	22.5	Length	0	45	97	173	226	.98**	2.9 $\pm$ .3
		Penetration	0	14	27	83	119		
	15	Length	0	52	70	92	124	.92*	2.0 $\pm$ .5
		Penetration	0	19	30	47	39		

\*\* Correlation coefficients significant beyond the 1% level.

\* Correlation coefficients significant beyond the 5% level.

With this information available, further experiments were conducted to determine the influence of relative humidity independent of its effect on growth. Inoculated leaves were placed over control solutions to give relative humidities of 90, 94, 96, 98, and 100%, and the control chambers maintained at 22.5° C. and illuminated for 12 hr. each day. In order to allow for approximately the same amount of growth at all humidities, leaves were fixed after various periods in the control chamber. About 40 hr. was allowed for those at relative humidity 100% and up to 80 hr. for those at 90%. The extent of growth was estimated by measuring the lengths of 100 germ tubes and taking the mean. The counts of penetrations and average lengths of germ tubes, together with the 'corrected' values for the counts are given in Table IV, the 'corrected' values being obtained by multiplying the observed number of penetrations by the factor 'average length of germ tube at relative humidity 100%/average length of germ tube at humidity concerned'. The results of the three experiments given in Table IV are shown graphically in Fig. 11. There is a striking increase in the frequency of penetration as the humidity decreases providing the same degree of growth is permitted. Over six times as many penetrations occurred per unit of growth at relative humidity 90% as at relative humidity 100%.

It may perhaps be suggested that the germ tubes that grow slowly may respond more to directive forces on the leaf than those that grow more rapidly, and that this may explain the results obtained. However, if the rate of growth had such an effect, the frequency of penetration at low temperatures where growth is reduced should be greater than that at higher, and the value

TABLE IV  
INFLUENCE OF HUMIDITY ON FREQUENCY OF PENETRATION

Relative humidity	No. of penetrations in 200 fields				Average length of 100 germ tubes in $\mu$				Penetration corrected for extent of growth			
	Expt.				Expt.				Expt.			
	1	2	3	4	1	2	3	4	1	2	3	4
100	79	76	348	98	70	97	140	160	79	76	348	98
98	172	170	489	154	70	86	160	115	172	192	392	221
96	297	235	870	146	65	81	160	92	319	282	690	257
94	259	363	1116	142	54	65	145	—	336	544	1070	—
90	624	207	1277	14	65	41	102	—	676	495	1750	—

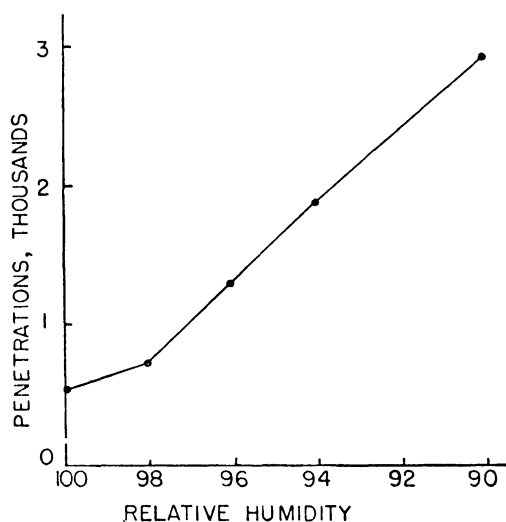


FIG. 11. The effect of relative humidity on frequency of penetration. The graph is a summary of Experiments 1, 2, and 3 of Table V and each point represents the 'corrected' value for number of penetrations in 3600 fields on 18 leaflets.

for the regression coefficients for growth and penetrations (Table III) at 15° should be greater than those at 22.5°. This is not the case. The effect of temperature in these series might possibly be such that a tendency for decreasing rate of growth to increase the frequency of penetration was exactly counteracted, but this is extremely unlikely. We may therefore conclude that the rate of growth is not a factor and that the results of the humidity experiments of Table IV show the influence of relative humidity on frequency of penetration, independent of any effect due to rate of growth.

In addition to these experiments on the effect of constant humidity on frequency of penetration, several were done in which the number of penetrations occurring in constant saturation and in fluctuating humidity were compared. These were modelled somewhat after Bond's (2) on *Cladosporium*

*fulvum* and consisted of placing inoculated leaves in large jar moist chambers in the greenhouse. Half of the leaves were maintained in a saturated atmosphere throughout the experiment, and the other half in a saturated atmosphere during the night and in one of 90% relative humidity during the day. Results of three such experiments are given in Table V.

It was found that over three times as many penetrations occurred in fluctuating humidity as in constant saturation. These results agree with Bond's and support the hypothesis that germ tubes enter in greater numbers when there is a water vapour gradient around the stomata.

TABLE V  
FREQUENCY OF PENETRATION OF *C. album* IN ATMOSPHERE OF  
CONSTANT SATURATION AND FLUCTUATING HUMIDITY

Experiment	Number of penetrations in constant saturation	Number of penetrations in fluctuating humidity
	79	319
	14	52
	107	300

The results of all the humidity studies are consistent in supporting the theory that water vapour attracts the germ tubes to the stomata. It may be felt that the differences of relative humidity are too small to make a definite reaction probable. However, Clayton (4) has shown that under carefully controlled conditions many fungus spores are extremely sensitive to small changes in humidity. This would support the contention that fungi may react to a small gradient around the stomata.

Acceptance of the importance of hydrotropism in infection does not preclude the possibility that other factors may also exert a directive influence on the germ tube. If the direction of growth of the hyphae on the leaf were controlled entirely by gradients of water vapour, then no penetrations should occur at relative humidity 100%, since the air in the leaf must be slightly less than saturated. Experiments reported under the study of stomata showed that leaves whose petioles were not supplied with water became somewhat flaccid even in a 'saturated' atmosphere and it may be that the actual value of the relative humidity of the air over pure water in these experiments sometimes dropped enough to give a gradient around the leaf. It is also possible that the penetrations that occurred were purely chance. On the other hand, there may be other substances diffusing from the stomata to which the fungus is positively tropic and this seems the more probable explanation. It is concluded that germ tubes of *C. album* are attracted to the stomata of sweet pea by a positive water vapour gradient and may be attracted by other factors. There is evidence presented above, and in Bond's (2) work, that this also applies to *C. fulvum*.

### Host Range

A number of leguminous plants was tested for susceptibility to *C. album*. This was done by inoculating sweet peas and the species to be tested at the same time and placing the plants in a moist chamber until the sweet peas showed distinct symptoms of disease. All were then examined and areas that showed any indication of disease were cleared in lactophenol and examined under the microscope. Each species was tested on at least two occasions. The following are the species that were tested:

*Lathyrus latifolius* L.  
*Vicia faba* L.  
*Vicia villosa* Roth.  
*Melilotus alba* Desr.  
*Medicago sativa* L.

*Pisum sativum* L.  
*Phaseolus vulgaris* L.  
*Trifolium pratense* L.  
*Glycine* Max Merr.

Penetrations were observed in a number of these species, viz. *L. latifolius*, *M. sativa*, *P. sativum*, *T. pratense*, and no doubt occurred in others.

The relative frequency of penetration into pea and sweet pea was estimated by placing inoculated leaves over a sugar solution to give relative humidity 94% and by counting penetrations in 200 fields per leaflet. The results showed 277 penetrations on eight leaflets of pea and 365 on eight leaflets of sweet pea. More penetrations occurred on sweet pea but its rougher leaves appeared to retain more spores, so that the difference cannot be considered significant. Apparently *C. album* may penetrate leaves that it does not infect with approximately the same frequency as those that it does. Such behaviour on the part of parasitic fungi has long been known for the rusts (Gibson (8), Hanes (10) ), and has also been shown for certain cases of direct penetration (Johnson (16) ). These studies on *C. album* again emphasize that penetration is not a function of a particular host-parasite reaction. While they do not in themselves support the idea that hydrotropism is an important factor in penetration, they indicate that some such non-specific factor or factors are of major importance.

In none of the plants tested did the fungus develop sufficiently to be considered as pathogenic although, on one occasion, appreciable development took place in red clover and, on another occasion, several places were found where the fungus had spread rather widely through a leaf of *P. sativum*. Attempts to duplicate these results were unsuccessful. The lesions were barely visible, and were shown to be caused by the fungus only after microscopic examination. Isolation was therefore impossible. It may be that a race having some potentialities to parasitize *P. sativum* was responsible for these cases, though Dowson found that neither *P. sativum* nor *Lathyrus aphaca* was attacked by the culture he studied. However, Hansford (11) reports *C. album* on bean and pea and again (12) on bean and vetch in Uganda, and Pape and Englehardt (20) report an attack on greenhouse vetches in Germany. It appears that within the species *C. album* there may be races pathogenic to leguminous plants other than the sweet pea.



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# STUDIES ON *FUSARIUM CULMORUM* BLIGHT OF CRESTED WHEAT AND BROME GRASS SEEDLINGS<sup>1</sup>

BY JOHN T. SLYKHUIS<sup>2</sup>

## Abstract

A number of pathogenic fungi were isolated from blighted brome grass and crested wheat grass seedlings grown in Saskatchewan and Ontario soils. The parasitism of one of the widely distributed and commonly occurring of these, *Fusarium culmorum* (W. G. Sm.) Sacc., was studied in more detail.

One per cent of *F. culmorum* sand-cornmeal inoculum caused more blight of brome grass seedlings in sterilized soil than did 6% in unsterilized soil. The development of *F. culmorum* in sterilized soil was optimum at 25° C. and declined rapidly with rising and more slowly with falling temperatures. Seedling blight was severe in sterilized soil at all temperatures from 10° to 35° C., but was significantly more severe near the optimum for the fungus provided the soil was not infested too heavily. In unsterilized soil, however, both the development of *F. culmorum* and the incidence of seedling blight were much greater at 10° to 20° C. than at 25° C. and higher, whereas other soil fungi and bacteria were more numerous at 25° C. and above than at the lower temperatures.

An infusion of unsterilized soil, a suspension of miscellaneous soil bacteria, and a mixture of 75 soil fungi suppressed the development of *F. culmorum* in sterilized soil, and also caused reductions in seedling blight. Of 136 soil fungi tested, only three reduced fusarial blight in sterilized soil. These antagonistic fungi included isolates of *Acremonium*, *Gliocladium fimbriatum* Gilman and Abbott, and *Phialophora*. Their ability to reduce disease incidence was not consistently correlated with the production of toxic filtrates, or the inhibition of *F. culmorum* in culture or in the soil but it was related to the effect they had on the development of *F. culmorum* in the environment in the immediate vicinity of the germinating seeds. This zone within which the germinating seed induces a characteristic change in the microbiological balance is designated as the 'spermatosphere'.

Disease incidence varied among different unsterilized field soils uniformly infested with *F. culmorum* and in these experiments was more severe in clay than in the soils of lighter texture. There was no consistent correlation between the suppression of blight and the numbers of fungi, bacteria, or actinomycetes in the different soils, but there was a correlation with the numbers of bacteria in the spermatosphere.

## Preliminary Survey of Grass Parasites

In view of the increasing importance of forage grasses in prairie agriculture, the diseases that may reduce their stand, yield, or longevity, or have an effect on other crops in the same rotation, assume a new significance. Parasites of the seedling stage are of such potential significance that studies were undertaken to gain further information on the diseases of grass seedlings and on certain factors influencing the activity of the associated pathogens that are harboured in the soil. Since crested wheat grass (*Agropyron cristatum* (L.) Gaertn.) and brome grass (*Bromus inermis* Leyss.) are the most important of the cultivated forage grasses in the Canadian prairies at the present time, these two were selected as hosts in the experimental work.

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When these grasses were sown in soil obtained from fields in various districts of central Saskatchewan, emergence was generally lower than in sterilized soil, and varying percentages of the seedlings that emerged died later. To discover the cause of this, fungi were isolated from well-washed seedlings that had been killed or damaged at various stages of development. The pathogenic fungi thus obtained included isolates of *Fusarium arthrosporioides* Sherb., *F. culmorum* (W. G. Smith) Sacc., *F. Equiseti* (Corda) Sacc., *F. oxysporum* Schlecht, *F. Scirpi* var. *acuminatum* (Ell. and Ev.) Wollenw., *F. Solani* (Martius) Appel. and Wollenw.,\* *Helminthosporium sativum* P.K. and B., *Pythium debaryanum* Hesse, *P. ultimum* Trow., and two isolates whose identity was not determined because they did not sporulate. All of the above-mentioned pathogens reduced emergence. *Pythium* species caused a certain amount of damping-off while some of the *Fusarium* isolates caused wilting and death, and often a brownish discoloration of the stem bases of seedlings. *H. sativum*, in addition to causing wilting and death of emerged seedlings, produced dark brown to black lesions on the stem of the seedling that resulted in stunting if not in killing the young plant. In addition this fungus occasionally caused dark lesions on the leaves, but the plants survived despite this type of injury. A comparison of the stands of crested wheat grass seedlings two weeks after sowing in sterilized soil infested with a number of pathogenic isolates is given in Table I.

TABLE I

THE INFLUENCE OF PATHOGENIC FUNGI ON THE STAND OF CRESTED WHEAT GRASS SEEDLINGS IN STERILIZED SOIL

Inoculum	Number of isolates tested	Average stand, %
Check	—	91
<i>Fusarium arthrosporioides</i>	1	58
<i>F. culmorum</i>	5	9
<i>F. Equiseti</i>	3	70
<i>F. oxysporum</i>	1	72
<i>F. Scirpi</i>	2	7
<i>F. Solani</i>	1	50
<i>Helminthosporium sativum</i>	16	12
<i>Pythium debaryanum</i>	4	56
<i>P. ultimum</i>	2	71
Unidentified isolate	1	64

\* The species of *Fusarium* were identified by Dr. L. W. Gordon, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

Pre-emergence blight caused by these pathogenic fungi was about equally severe in crested wheat grass and brome grass, but under the conditions of these experiments postemergence blight was more severe in crested wheat grass than in brome grass. Later experiments showed that susceptibility to the latter type of blight was influenced by temperature. Both grasses were more susceptible at temperatures of 25° C. and higher than at the lower

temperatures, and although there was little difference in susceptibility of the two grasses at the higher temperatures, brome grass was less susceptible than crested wheat grass at moderate and low temperatures.

Isolations from crested wheat and brome grass seeds yielded a large number of various fungi, among which were pathogenic isolates of *F. culmorum*, *F. Equiseti*, *F. Scirpi* var. *acuminatum*, and *II. sativum*. Further isolations were made from 39 samples of brome grass and 35 of crested wheat grass seeds grown in widely scattered areas of Saskatchewan and Manitoba in 1942. *II. sativum* was detected in only two samples of brome and five of crested wheat grass seeds. *Fusarium* isolates were obtained from two samples of brome and 21 of crested wheat grass seeds but not all of these isolates were pathogenic.

### The Parasitism of *Fusarium culmorum*

It was the purpose of the experiments that follow to study some of the factors that influence seedling blight caused by *F. culmorum*, one of the more important and widely distributed pathogens of cereal and forage grass seedlings, especially on the Canadian prairies. These studies have dealt chiefly with the influence of temperature and of soil microflora on disease incidence.

#### A. The Survival and Virulence of *Fusarium culmorum* in Sterilized and Unsterilized Soil

The virulence of *F. culmorum* was compared in sterilized and unsterilized Saskatchewan black loam soil. Inoculum was prepared by growing *F. culmorum* for one month in flasks containing a sterilized moist mixture of sand and 2% cornmeal by weight. This inoculum was mixed with quantities

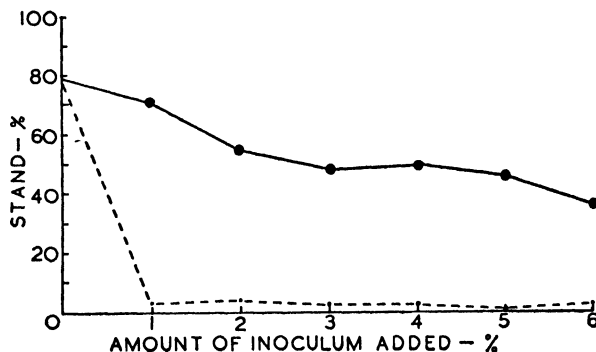


FIG. 1. Influence of the amount of *F. culmorum* inoculum on seedling blight of brome grass in sterilized and unsterilized portions of a Saskatchewan black loam soil. ——— Unsterilized. - - - - - Sterilized.

of each soil at the rates of 0, 1, 2, 3, 4, 5, and 6% by weight, and two days later 50 brome grass seeds were sown in two 6 in. pots of each treatment. The percentage stand of seedlings two weeks after sowing is plotted in the graph in Fig. 1 against the percentage of inoculum in the test soils. The stands.

were about equal in the two soils in the absence of inoculum. When the sterilized soil was infested with *F. culmorum* the stand was drastically reduced, and 1% of inoculum reduced seedling survival as effectively as larger amounts. In the unsterilized soil, on the other hand, 1% of inoculum reduced the stand only slightly, and greater concentration of inoculum caused greater reductions in stand, but even 6% of inoculum in unsterilized soil reduced the stand less than half as much as did 1% in sterilized soil. This suggests that biotic factors are exerting a strong influence in unsterilized soil.

Further evidence of the importance of the biotic factors in the soil was supplied by other experiments, which showed that seedling blight was reduced by the addition of well-rotted manure to sterilized and unsterilized soil infested with *F. culmorum* inoculum (13). Also, when sterilized and unsterilized soils were re-sown five months after infestation with *F. culmorum*, there was no seedling blight caused by this fungus. Evidently under the conditions of the experiment the *F. culmorum* added with the inoculum was unable to establish itself at the high concentration among the normal soil flora, and was crowded out by the micro-organisms that repopulated the sterilized soil.

In another experiment the stands of brome grass seedlings in freshly sterilized and unsterilized soil infested with 6% *F. culmorum* inoculum were compared with the stand in similarly infested pots of soil that had been sterilized one year earlier and allowed to stand in the greenhouse. The results (Table II) showed that as usual the stands in the freshly sterilized soil were much lower than in the unsterilized soil equally infested with *F. culmorum*.

TABLE II

THE RELATION BETWEEN MICRO-ORGANISM POPULATIONS AND SEEDLING STANDS  
IN UNSTERILIZED AND REPOPULATED STERILIZED SOILS  
UNIFORMLY INFESTED WITH *F. culmorum*

Soil	Stand, %	Fungi/gm.	Bacteria/gm.
Freshly sterilized	6	.	
Sterilized 1 year previously	46	1,650,000	12,670,000
Unsterilized	36	100,000	4,810,000

In the soil that had been sterilized one year earlier, however, the stand was 10% higher than in the unsterilized soil. The numbers of bacteria and fungi populating the old sterilized and unsterilized soils were investigated by the dilution plate method, using peptone-glucose acid agar for the fungi and sodium albuminate agar for bacteria (3). The counts showed that the bacteria were about three times and the fungi about 16 times more abundant in the old sterilized than in the unsterilized soil. This suggests that the suppression of seedling blight caused by *F. culmorum* may be a function of the number of micro-organisms populating the soil.

The survival of *F. culmorum* in sterilized soil watered with sterile water and with an infusion of unsterilized soil was investigated. Three textures of soil, clay loam, loam, and sandy loam were used. One hundred and fifty grams of each type of soil was put into each of two 300 cc. Erlenmeyer flasks, which were then plugged with cotton and sterilized for two hours at 15 lb. pressure. Two days after sterilization each flask was inoculated with 10 cc. of a suspension of *F. culmorum* spores in sterile water. To one flask of each type of soil 10 cc. of sterile water was added, and to the other 10 cc. of an infusion made by shaking 5 gm. of a Saskatchewan loam soil with 100 cc. of sterile water, then allowing the heavy soil particles to settle, and pouring off the liquid. The flasks were left undisturbed for 24 hr., then shaken vigorously to mix the soil thoroughly. At this time counts were made by the dilution plate method of the numbers of *F. culmorum* isolates per gram. The flasks were kept at room temperature and further counts were made 7, 23, and 55 days after inoculation. Since the numbers of isolates from the different soils similarly treated were not very different, the results given in Table III are the averages of the counts from the three soils.

TABLE III  
THE INFLUENCE OF AN INFUSION OF UNSTERILIZED SOIL ON  
THE DEVELOPMENT OF *F. culmorum* IN STERILIZED SOIL

Days after inoculation	<i>F. culmorum</i> isolates per gram after the addition of:	
	Sterile water	Soil infusion
1	11,600	7900
7	94,100	36,600
23	124,300	39,300
55	47,200	13,000

In both series of flasks the numbers of isolates were highest in the counts made on the 23rd day after inoculation, and were greatly reduced by the 55th day. The latter reduction may have been accentuated by the slow drying out of the soil. The most significant feature of the results, however, was that on and after the seventh day the numbers of *F. culmorum* isolates were much higher in the soils to which sterile water had been added than in those moistened with the infusion of unsterilized soil. Colonies of soil fungi were frequent on the medium on which dilutions of the latter soils were plated for counting *F. culmorum* isolates, and when dilutions were plated on sodium albuminate agar it was found that on and after the seventh day there were more than twenty million bacteria per gram. Evidently the development of fungi and bacteria, which were added with the unsterilized soil infusion, suppressed the development of *F. culmorum*.

Experiments with suspensions of miscellaneous soil bacteria and with mixtures of 75 miscellaneous soil fungi (13) have shown that when added to

sterilized soil infested with *F. culmorum*, both bacteria and fungi retard the development of the pathogen in the soil and reduce the incidence of seedling blight. It is therefore likely that the suppression of seedling blight in artificially infested unsterilized soil is a result of the suppression of the development of *F. culmorum* by the micro-organisms inhabiting the soil.

## B. The Relation of Temperature to the Development and Pathogenicity of *Fusarium culmorum*

### 1. In Sterilized Soil

The influence of temperature on the development of *F. culmorum* in sterilized soil was investigated. One-hundred-gram quantities of black loam soil were autoclaved in 250 cc. Erlenmeyer flasks. To each, 15 cc. of a heavy suspension of *F. culmorum* spores was added, then these flasks of inoculated soil were incubated at six temperatures ranging from 10° to 35° C. Special precautions were taken to reduce evaporation and the flasks were shaken periodically to mix the soil thoroughly. After 12 days' incubation the numbers of *F. culmorum* isolates per gram of soil were counted by the dilution plate method. In another experiment the influence of temperature on the radial growth of colonies growing on an agar medium was investigated throughout a range of 5° to 40° C. The results of these experiments are presented in Fig. 2 from which it is apparent that the graphs obtained by the two methods were very similar. In both experiments development was greatest at 25° C., but with lowering temperatures it decreased gradually, while with rising temperatures it decreased more rapidly.

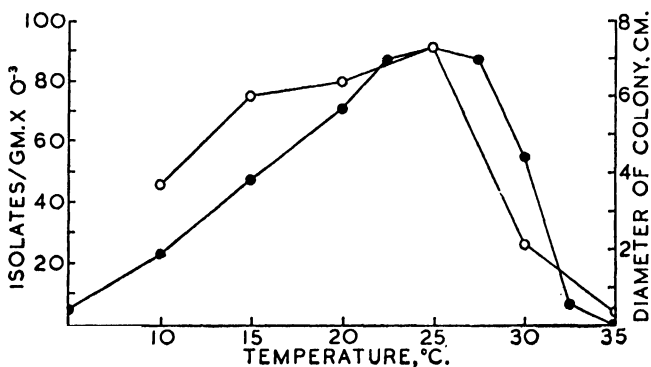


FIG. 2. Influence of temperature on *F. culmorum* development on agar and in soil.  
 ● = Radial growth after three days on potato dextrose agar. ○ = Isolates per gram of soil.

Wisconsin temperature tanks were used to study the influence of soil temperatures on the incidence of blight in brome and crested wheat grass seedlings. The six tanks, each of which was fitted with eight metal cans 18 cm. in diameter and 30 cm. deep, were adjusted to give soil temperatures ranging from 10° C. to 35° C. with 5° intervals. A steam sterilized mixture of three parts clay loam soil and one part sand was prepared. The soil for four cans in each tank was mixed in bulk with 1% of its weight in a

two week old culture of *F. culmorum* on a 2% cornmeal-sand mixture. The soil for the remaining four cans was not infested. Brome grass was sown in two cans of infested and two of uninfested soil in each tank, and crested wheat grass was sown in the others at the rate of 100 seeds per can. The moisture content of the soil was held near 40% of the moisture holding capacity. The development of seedlings was observed and the stands were counted three weeks after sowing in three replications of this experiment.

Both grasses began to emerge four days after sowing at 30° and 35° C., but at lower temperatures the time for emergence was increased and was eight or nine days at 10° C. Growth was slower but the seedlings were hardier at temperatures up to 20° C., while at higher temperatures growth was more rapid and the seedlings appeared chlorotic and spindly, and even in the uninfested soil some of them died, especially at 35° C. The influence of temperature on the stands of the two grasses was very similar. The results with brome are presented graphically in Fig. 3. In uninfested soil the stand

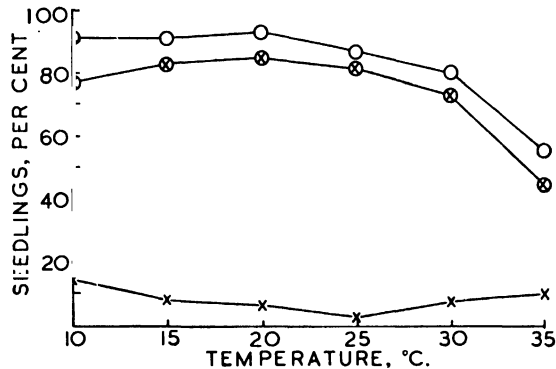


FIG. 3. Influence of temperature on blight and stand of brome grass seedlings in sterilized soil. ○ = Stand in uninfested soil. × = Stand in soil infested with *F. culmorum*. ⊗ = Blight caused by *F. culmorum*.

was uniformly high at temperatures up to 25° C., but at higher temperatures than this it was significantly lower. Thus, although high soil temperatures increased the rate of emergence and growth, low temperatures were more favourable for high stands of vigorous seedlings. In soil infested with *F. culmorum* the stand was low at all temperatures but was lowest at 25° C.

The amount of seedling blight caused by *F. culmorum* is indicated by the differences between the stands in infested and uninfested soils, and was greatest at temperatures of 15° to 25° C., the temperature range most favourable for the development of grass seedlings, and also for the development of *F. culmorum* in sterilized soil (Fig. 2). It appears, therefore, that in sterilized infested soil the incidence of seedling blight at different temperatures depends largely on the relative concentration of *F. culmorum* that develops in the soil. This was borne out in other temperature experiments in which higher rates of infestation of sterilized soil were used. With such higher rates of infestation



no seedlings survived except a small percentage at 30° and 35° C., hence the effect of temperature was largely obscured because of the high concentration of *F. culmorum* at all temperatures.

## 2. In Unsterilized Soil

In a preliminary experiment on the influence of temperature on blight of brome grass seedlings in unsterilized soil, a Saskatchewan black loam soil infested with various amounts of *F. culmorum* inoculum was used. The Wisconsin temperature tanks were employed to maintain six temperatures ranging from 10° to 35° C. Two cans in each tank contained soil infested with 4, 7, and 10% of inoculum while two others contained uninfested soil. One hundred brome grass seeds were sown in each can. The soils were kept moderately moist by frequent light sprinklings. The seedlings were counted 10 days after first emergence in each tank.

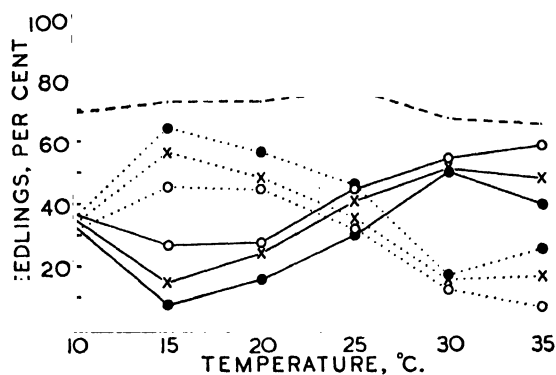


FIG. 4. The relation of temperature to disease incidence in unsterilized soil infested with different amounts of *F. culmorum* inoculum (O = 4% inoculum, X = 7% inoculum, ● = 10% inoculum). — Stand in infested soil. .... Seedling blight caused by *F. culmorum*. - - - - - Stand in uninfested soil.

Fig. 4 shows the graphs of the stands of seedlings at different temperatures in the uninfested soil and in the soil infested at different rates with *F. culmorum*. In the uninfested soil the stand was slightly higher at 25° C. than at the higher and lower temperatures. In the infested soils, at every rate of infestation, the stands were lowest at 15° to 20° C., and highest at 25° to 35° C. The differences between the stands in the infested and uninfested soils indicate the amount of seedling blight caused by the *F. culmorum* added to the soil. At every temperature the severity of blight was related to the amount of inoculum that had been added to the soil, but the form of the graphs of the effect of temperature on blight was similar at every rate of infestation. In each instance blight was most severe at 15° C. but decreased with increasing temperatures to a minimum at 30° to 35° C. This is in striking contrast with results in sterilized soil where disease incidence was greatest at 25° C., which likewise is the optimum temperature for the development of the pathogen.

The influence of temperature on seedling blight and on the numbers of bacteria and fungi in two other Saskatchewan soils artificially infested with 4% *F. culmorum* was investigated in the same manner. They were black parkland soils, one a clay loam, the other a sandy loam, and both had grown cereal crops for a number of years.

Although the stands were consistently lower in the infested clay loam than in the sandy loam soil, the graphs of the influence of temperature on stand were similar in form for both. The average of the results from the two soils is presented in Fig. 5. In the uninfested checks the stand was slightly higher at 25° C. than at either higher or lower temperatures. In the infested soil the stand was lowest at 15° C. and increased with rising temperatures to a maximum at 35° C. The incidence of seedling blight thus was greater at 15° C. than at the higher temperatures.

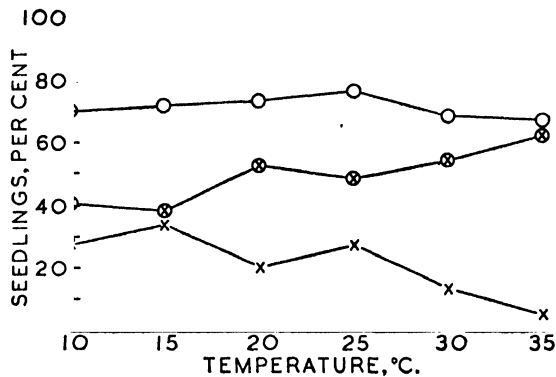


FIG. 5. The influence of temperature on percentage stand and seedling blight of brome grass in Saskatchewan field soil not infested and infested with *F. culmorum*. ○ = Stand in uninfested soil. × = Stand in infested soil. ⊗ = Seedling blight in infested soil.

The numbers of micro-organisms in the above two soils were counted after they had been kept in the tanks at the six temperatures for 90 days after infestation. The counts were made from dilution plates using sodium albuminate agar for the bacteria and peptone glucose agar for the fungi. Other fusaria were negligible in the soils and since *F. culmorum* colonies could be distinguished from those of other fungi, all were counted on the same plates. The counts of these micro-organisms for the two soils were averaged and the graphs of the results are shown in Fig. 6. *F. culmorum* isolates were most abundant at 15° C. and slightly less at 10° C. The numbers decreased with increasing temperatures to a minimum at 35° C. There was no marked influence of temperature on the numbers of other fungi in the soil. The bacteria, however, were significantly more numerous in the soils at 20° C. to 35° C. than at 10° and 15° C. Hence, in these soils, three months after being infested with *F. culmorum*, bacterial numbers were greater at the higher temperatures while the numbers of *F. culmorum* isolates were higher at the lower temperatures where seedling blight was most severe.

The influence of temperature on seedling blight and the numbers of micro-organisms in infested Ontario soils was likewise investigated. Four soils collected near Vineland, Ont. were selected to represent different textures and

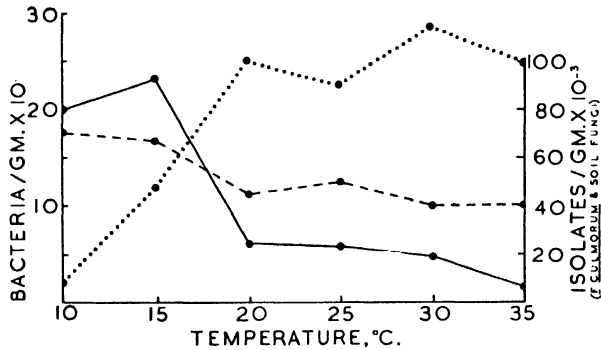


FIG. 6. The populations of bacteria, fungi, and *F. culmorum* in Saskatchewan field soil. (*F. culmorum* inoculum was added to the soil three months previously and the soils were kept at different temperatures during that time.) ——— *F. culmorum*. ---- Fungi. .... Bacteria.

cultural histories. One was clay, the other three were loam. The clay and one loam were from a field growing an alfalfa-timothy mixture. Another loam was from an oat field, and the third was from grass sod. The soils were infested with 4% *F. culmorum* inoculum, and two cans of each were included in each temperature tank and 100 brome grass seeds were sown per can. The experiment was repeated and the results averaged. Although blight was consistently more severe in the clay than in the other soils, the forms of the graphs of the influence of temperature on blight were similar for all, hence the results were averaged and the graph so obtained is presented in Fig. 7. At the conclusion of the second experiment to determine seedling

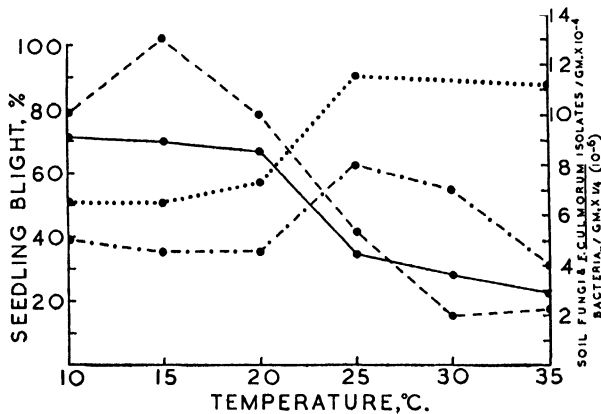


FIG. 7. The effect of soil temperature on numbers of *F. culmorum* isolates, other fungi, and bacteria, and on blight of brome grass seedlings in Ontario soil infested with 4% *F. culmorum* inoculum. ---- *F. culmorum*. .... Soil fungi. .... Bacteria. ——— Blight.

blight, which was 40 days after infestation, counts were made of the numbers of micro-organisms in the soils. The results from the four soils were averaged, and the resulting graphs of the numbers of *F. culmorum* isolates, fungi, and bacteria are presented, with the graph of seedling blight, in Fig. 7.

In these experiments with infested Ontario soils, as was the case also with Saskatchewan soils, seedling blight was more severe at the lower (10° to 20° C.) than at the higher temperatures (25° to 35° C.). Similarly the development of *F. culmorum* was greater at the lower than at the higher temperatures while bacteria were more abundant at the higher temperatures. In this experiment with Ontario soils the fungi too were somewhat more abundant at 25° to 30° C. than at lower temperatures.

These experiments on the influence of temperature on the incidence of seedling blight and the development of *F. culmorum* have revealed sharp contrasts between sterilized and unsterilized soils. In sterilized soil both disease incidence and the development of the pathogen were optimum at 25° C., but in unsterilized soils they were greater at much lower temperatures. On the other hand the development of bacteria, and in some instances of soil fungi, was greatest at and above the temperature that is optimum for *F. culmorum* development in sterilized soil. These results indicate that the incidence of blight is dependent on the development of *F. culmorum* in the soil, which in turn is greatly influenced by soil micro-organisms.

### C. *The Antagonistic Effects of Certain Soil Fungi to F. culmorum*

From a large number of fungi isolated from an Ontario loam soil and from a Saskatchewan loam from a field that had grown cereals for a number of years tests were made for antagonism to *F. culmorum* with 110 isolates from the Saskatchewan and 26 from the Ontario soil. The influence of isolates of the soil fungi on the pathogenicity of *F. culmorum* was tested in sterilized soil infested with 2% of *F. culmorum* soil – oat hull inoculum. Enough of this soil for two 5 in. pots was infested with 2% or more of the inoculum of an isolate to be tested, and two days later 25 grass seeds were sown in each pot. Antagonism to *F. culmorum* was indicated if an isolate increased the percentage of seedlings that survived.

The influence of these isolates on the growth of *F. culmorum* on an agar medium was also studied. A piece of agar cut from the edge of a growing culture of the isolate to be tested was placed 3 cm. from a similar piece of *F. culmorum* inoculum on potato dextrose agar in a Petri dish. After five to six days' incubation at 25° C. the association effects of the isolate and *F. culmorum* were observed for indications of antagonism.

On the basis of these preliminary tests, five isolates were selected for further studies. Three were selected because they increased the survival of grass seedlings in soil infested with *F. culmorum*. Two of these, *Gliocladium fimbriatum* Gilman and Abbott, and an *Acremonium* species, were from the Saskatchewan soil. The third, isolated from Ontario soil, belongs to the genus *Phialophora*. Isolates of *Trichoderma lignorum* (Tode) Harty and

*Penicillium urticae* Bain\* were also selected because of their antagonism to *F. culmorum* on agar medium.

1. *The Influence of Selected Soil Fungi on the Incidence of Seedling Blight Caused by F. culmorum*

The influence of each of the five selected soil isolates, and of a mixture of 75 isolates including the latter five, on the stands of crested wheat grass and brome grass seedlings in sterilized soil uniformly infested and not infested with *F. culmorum* is shown in Table IV. For comparison the stands of brome

TABLE IV

THE INFLUENCE OF SOIL FUNGI ON THE STANDS OF CRESTED WHEAT AND BROME GRASS SEEDLINGS IN INFESTED SOIL

Inoculum added to sterilized soil	Stand, %			
	Crested wheat grass		Brome grass	
	Check	<i>F. culmorum</i>	Check	<i>F. culmorum</i>
Check	92	13	90	16
<i>Phialophora</i>	98	92	90	92
<i>Acremonium</i>	—	—	90	68
<i>Gliocladium</i>	82	76	88	68
<i>Penicillium</i>	92	14	86	12
<i>Trichoderma</i>	88	12	88	10
Mixture of 75 isolates	—	—	86	24
Unsterilized soil	—	—	84	68

grass seedlings in equally infested and uninfested unsterilized soil are also given. Inasmuch as none of the soil fungi was pathogenic, they caused no significant reduction in seedling stand in sterilized soil, whereas *F. culmorum* reduced the stand severely. The addition of *Phialophora* to soil infested with *F. culmorum* increased the stands of seedlings to equal that in uninfested soil. Similarly *Acremonium* and *Gliocladium* caused increases in stand. The increases caused by these fungi were less spectacular, but the stands in these instances were equal to the stand in unsterilized soil that was equally infested with *F. culmorum*. Neither *Penicillium* nor *Trichoderma* caused any increase in seedling survival in infested soil. A mixture of 75 isolates of soil fungi, including the above five, caused only a slight increase in seedling survival as compared with unsterilized soil.

2. *The Influence of Rates of Infestation, Soil Acidity, and Temperature on the Suppression of Pathogenicity by Soil Fungi*

It has been shown above that as the amount of *F. culmorum* inoculum added to unsterilized soil was increased the stand of healthy seedlings decreased. In a similar experiment conducted with sterilized soil infested with selected soil fungi, it was shown that as the rate of infestation with a

\* Identified by Dr. K. B. Raper, Acting Head, Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill.

2% cornmeal-sand inoculum of *F. culmorum* was increased from 1 to 2, 4, and 8%, the stands of seedlings in sterilized soil to which *Phialophora* had been added decreased from 45 to 35, 15, and 3%. There were indications of a similar effect of increased concentrations of inoculum in soil to which *Gliocladium* and *Acremonium* had been added, but the virulence of the *F. culmorum* inoculum was very high and suppressive effects of the soil fungi were obscured at a lower level of infestation with *F. culmorum* inoculum.

Experiments have shown that there may be considerable variation in the virulence of different preparations of inoculum. This observation was also made by Tyner (14). Since results vary with different rates of infestation the results of different experiments with the same isolates of fungi but with different preparations of inoculum would also be expected to vary. Greater increases in seedling stand resulted from the addition of *Phialophora* to sterilized soil infested with *F. culmorum* in experiments in which soil-oat hull medium was used to culture the fungi than when sand-cornmeal inoculum was used. Such differences have been attributed to a higher virulence of the sand-cornmeal inoculum, and hence a higher concentration of *F. culmorum* than was obtained by adding soil-oat hull inoculum to the soil. *Gliocladium* and *Acremonium* are less effective than *Phialophora* in reducing the incidence of blight caused by *F. culmorum*, and their effect is obscured with lower concentrations of *F. culmorum* in the soil, hence if the virulence of the *F. culmorum* inoculum were too high, or the amount added to sterilized soil were too great, no increase in seedling stand would be obtained by the addition of *Acremonium* or *Gliocladium* to the soil.

Different soils and mixtures of soils were sterilized, then the pH values were determined with a Leeds Northrup glass electrode pH metre. The pH values were as follows: a muck type of soil, 7.6; a mixture of loam and sand, 7.2; a mixture of loam and peat moss, 6.8; and peat moss, 5.2. The stands of brome grass seedlings obtained when these soils were not infested did not vary significantly. Similarly the stands were low in all soils infested with *F. culmorum* with no significant differences between the soils. However, in soils infested with *F. culmorum* and *Phialophora* the stands were as high as 100% in the muck soil of pH 7.6 and as low as 30% in the peat moss of pH 5.2, while in the soils with intermediate pH values, the stands were intermediate. *Phialophora* was therefore more effective in suppressing pathogenicity in the more alkaline than in the acid soils. Although the influence of soil reaction on *Acremonium* and *Gliocladium* was not as striking as that on *Phialophora* in this experiment, because of low stands in all soils, these fungi also suppressed the pathogenicity of *F. culmorum* most in the alkaline soils. *Trichoderma* and *Penicillium* were not effective in any of the soils.

The influence of soil temperatures on the antagonistic effects of the five selected isolates was compared. Wisconsin temperature tanks were used to regulate the soils at temperatures of 15°, 20°, 25°, 30°, and 35° C. One can in each tank contained unsterilized loam soil infested with 2% of *F. culmorum* inoculum. All the other cans contained sterilized soil, and all but one in each

tank was infested with 2% of *F. culmorum* inoculum. To individual cans of soil thus infested, 2% of the soil-oat hull inoculum of the soil fungi was added. The infested soil in one can was not infested with a soil fungus. One hundred brome grass seeds were sown in each can and the soil moisture was held at about 40% of the moisture holding capacity. The stands of seedlings in each soil at the various temperatures are presented in graph form in Fig. 8.

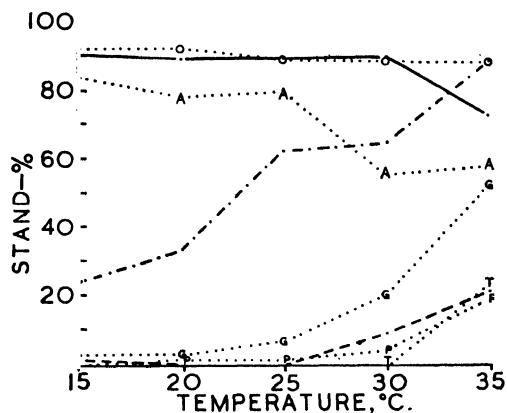


FIG. 8. The relation of temperature to the stands of brome grass seedlings in soil infested with *F. culmorum* and certain soil fungi. — Sterilized soil, uninfested. --- Sterilized soil, infested with *F. culmorum*. .... Sterilized soil infested with *F. culmorum* and a soil fungus. A = *Acremonium*. O = *Phialophora*. G = *Gliocladium*. P = *Penicillium*. T = *Trichoderma*. - - - - - Unsterilized soil infested with *F. culmorum*.

The stands in sterilized soil infested with *F. culmorum* were 1 to 0% at temperatures of 15° to 25° C., but increased to 9% at 30°, and to 21% at 35° C. Neither *Penicillium* nor *Trichoderma* caused any increase in stand at any temperature whereas *Phialophora* increased the stand to at least the equal of that in uninfested sterilized soil at all temperatures. *Acremonium* caused a greater increase in stand at the lower than at the higher temperatures, while *Gliocladium* on the other hand, was most effective at the higher temperatures. In unsterilized soil infested with *F. culmorum* the stands were low at the lower temperatures but increased significantly toward higher temperatures. These antagonistic effects to *F. culmorum* in unsterilized soil show a similar relation to temperature in this experiment as has been illustrated in experiments described above, but in comparing this with the various responses to temperature of the individual competitive fungi, it is interesting to note that the antagonistic effects of *Phialophora* were not appreciably influenced by soil temperature, while those of *Acremonium* were greatest at the lower, and those of *Gliocladium* greatest at the higher temperatures.

### 3. The Relation Between the Suppression of Fusarium Blight by Certain Soil Fungi and Their Antagonistic Effects in Culture

The influence of the soil fungi on the growth of *F. culmorum* in culture was investigated in comparison with their influence on seedling blight as shown above.

In one experiment the isolates were grown in apposition with *F. culmorum* on potato dextrose agar and on a soil - oat hull medium in Petri dishes, the inocula being placed about 3 cm. apart. The association effects and relative growth of *F. culmorum* and different soil fungi varied. Neither *Phialophora* nor *Acremonium* made very extensive growth on agar for *F. culmorum* grew more rapidly and soon surrounded the colonies of the former fungi. On the soil medium, however, *Phialophora* grew more rapidly than and suppressed the growth of *F. culmorum*, while the growth of *Acremonium* was about the same on the soil as on the agar medium. *Gliocladium* produced colonies of limited size on both media while *F. culmorum* covered the remainder of the dish. On agar, however, *F. culmorum* grew close to but not in contact with *Gliocladium*. A similar but more pronounced effect was demonstrated by *Penicillium*, which produced a distinct zone of inhibition on agar, but on the soil medium *F. culmorum* grew in close contact with it. *Trichoderma* very effectively suppressed *Fusarium* development on agar for it quickly covered the medium and limited the development of the pathogen to a very small colony with limited aerial mycelium. On the soil medium *F. culmorum* developed more abundantly than *Trichoderma* and confined the growth of the latter to a sector of the medium. These results show that the association effects of these soil fungi and *F. culmorum* differ on different media. Furthermore the fungi that effectively reduced disease incidence were less effective in suppressing the pathogen on agar than other fungi that did not reduce the amount of blight.

In addition to the above experiments the relative growth of *F. culmorum* and the soil fungi was compared on two solid media and a liquid medium inoculated with mixed spore suspensions of the fungi. The two solid media were potato dextrose agar and the soil - oat hull medium described above. The liquid medium was a potato dextrose decoction. Spore suspensions in sterile water were made from the various fungi. An equal volume of a suspension of *F. culmorum* conidia was added to the spore suspension of each soil isolate. Petri dishes of the two solid media and 200 cc. Erlenmeyer flasks containing 25 cc. of the liquid medium were inoculated with the mixed suspensions. The resultant growth of *F. culmorum* and the soil fungi was similar on all media. *F. culmorum* and *Phialophora* made about equal growth, hence neither seemed to suppress the other very strongly if at all. *Fusarium* made only slight growth when mixed with *Gliocladium*, and no growth at all when mixed with *Trichoderma* and *Penicillium* while the latter fungi covered the entire medium in every instance. These results, like those on growth in apposition, show that *Phialophora*, which greatly reduces the pathogenicity of *F. culmorum*, does not effectively suppress its growth in culture, while other fungi that have less effect or no effect on pathogenicity strongly suppress growth in culture.

Additional experiments showed that when these soil fungi were grown on potato dextrose decoction for seven days, the filtrates of the media on which *Gliocladium*, *Trichoderma*, or *Penicillium* had grown were toxic to *F. culmorum*.



When nutrients were added, *Fusarium* grew abundantly on the filtrates from *Phialophora* and *Acremonium*, moderately on the filtrates of *Gliocladium* and *Trichoderma*, and not at all on the *Penicillium* filtrate. *F. culmorum* inoculum was soaked in filtrates of the various fungi before it was mixed in the soil. The filtrates of *Gliocladium* and *Penicillium* were the only ones that reduced the virulence of the inoculum, and of these *Penicillium* was the more effective. From the results it is concluded that the production of toxic filtrates by these soil fungi does not correlate with the ability of the fungi to suppress the seedling blight caused by *F. culmorum*.

#### 4. The Relation Between the Suppression of the Incidence of Blight by Certain Soil Fungi and the Suppression of *F. culmorum* Development in the Soil

Experiments were conducted to compare the influence of antagonistic soil fungi on the development of *F. culmorum* in the soil with their influence on the incidence of seedling blight. Sterilized soil was infested with *F. culmorum* soil - oat hull inoculum, and quantities of this infested soil sufficient for two 5 in. pots each were infested with *Phialophora*, *Acremonium*, *Gliocladium*, *Penicillium*, and *Trichoderma* isolates, while soil for two other pots was not further infested. Two pots of unsterilized soil were also infested with *F. culmorum* inoculum. Brome grass seeds were sown at the rate of 25 per pot and the resulting stands were counted two weeks later. The numbers of *F. culmorum* isolates per gram of each soil were also counted by means of the dilution plate technique.

The results (Table V) show that, with the exception of *Penicillium* and *Trichoderma*, the fungi tested significantly increased the stands of seedlings in infested soil. The extent of this increase was not related directly to the

TABLE V

THE INFLUENCE OF CERTAIN SOIL FUNGI ON THE STAND OF SEEDLINGS AND THE NUMBER OF *F. culmorum* ISOLATES IN INFESTED SOIL

Soil	Fungus added	Stand, %	<i>F. culmorum</i> isolates per gram
Unsterilized	—	52	33,000
Sterilized	—	4	94,000
"	<i>Phialophora</i>	82	71,000
"	<i>Acremonium</i>	54	76,000
"	<i>Gliocladium</i>	36	94,000
"	<i>Penicillium</i>	10	( <i>Penicillium</i> covered plate)
"	<i>Trichoderma</i>	2	( <i>Trichoderma</i> covered plate)

concentration of *F. culmorum*. In sterilized soil infested with *F. culmorum* the stand was low and the number of *F. culmorum* isolates was high, but with the addition of *Gliocladium* to otherwise similarly infested soil the stand was strikingly increased but there was no change in the numbers of isolates of the pathogen. Further, the addition of *Phialophora* to infested soil caused a remarkable increase in stand but only a slight decrease in the concentration of

*F. culmorum*, whereas *Acremonium* caused a less marked increase in stand but almost as great a suppression of the pathogen. Or again, the stands in unsterilized soil and in *Acremonium* infested sterile soil were almost identical, but there were more than twice as many isolates of *F. culmorum* per gram in the latter than in the former soil. This strongly suggests, therefore, that although these soil micro-organisms influence the development of *F. culmorum* in the soil to a certain degree, they must exert a more effective suppressive influence on the pathogen in some phase more closely associated with the seedlings. The developmental phases of the seedling were consequently studied next.

By the dilution plate method the numbers of *F. culmorum* isolates in the rhizospheres, and as well in the soil apart from the roots of brome grass seedlings, were counted 10 days after the seeds were sown in sterilized loam soil infested with 1% of *F. culmorum* sand-cornmeal inoculum and 2% of the inoculum of different soil isolates. In Table VI the stands of seedlings and the numbers of isolates in the variously infested soils are shown. The concentration of the pathogen was consistently greater in the rhizosphere than in

TABLE VI

THE INFLUENCE OF SOIL FUNGI ON THE STAND OF SEEDLINGS AND ON THE NUMBERS OF *F. culmorum* ISOLATES FROM THE RHIZOSPHERE 10 DAYS AFTER SOWING IN INFESTED SOIL

Inoculum	Stand, %	<i>F. culmorum</i> isolates per gram	
		Rhizosphere	Soil apart from roots
Check	13	16,000	8800
<i>Phialophora</i>	80	13,800	7800
<i>Acremonium</i>	37	16,900	11,800
<i>Gliocladium</i>	33	18,000	12,000

the soil apart from the roots, even where there was little seedling blight, and there was no consistent correlation between seedling stand and the numbers of isolates of the pathogen either in the rhizosphere of 10-day seedlings or in the soil apart from the roots of the seedlings.

Since the above comparisons relate to a stage of seedling development considerably older than that at which most of the blight occurs, another experiment was conducted in which counts were made of the numbers of isolates associated with seedlings at the time of emergence. Brome grass seeds were sown in pots of sterilized soil infested with 2% *F. culmorum* inoculum and 2% of the inoculum of the different soil isolates as well as in pots of infested unsterilized soil. Five days later when the seedlings were just starting to emerge, they were removed from the soil and, after shaking off the loose soil, the seedlings with the remains of the seeds attached were put immediately into flasks of sterile water and dilutions were made for plating.

In duplicate pots the seedlings were allowed to grow so that the stands in the infested soils could be counted. These stands and the numbers of isolates of *F. culmorum* counted in the soil apart from and associated with germinating seeds are given in Table VII.

TABLE VII

THE INFLUENCE OF SOIL FUNGI ON THE STAND OF SEEDLINGS AND ON THE NUMBER OF *F. culmorum* ISOLATES IN THE SOIL CLOSELY ASSOCIATED WITH GERMINATING SEEDS IN INFESTED SOIL

Inoculum	Stand in duplicate pots, %	<i>F. culmorum</i> isolates per gram of soil	
		Spermatosphere	Apart from seeds
Check	0	80,000	13,300
<i>Phialophora</i>	79	10,200	7400
<i>Acremonium</i>	0	66,600	13,800
<i>Gliocladium</i>	8	31,400	11,700
Unsterilized soil	56	12,600	12,500

The stands varied from 0% in the check to 79% with the addition of *Phialophora*. The unusually small increase in stand brought about by *Gliocladium*, and the absence of any increase with the addition of *Acremonium* is attributed to too low a concentration of these fungi in relation to the concentration of *F. culmorum* in the soil. As in the previous experiments, there was no consistent correlation between the numbers of isolates in the soil and the stands of seedlings. The numbers of isolates in close association with the germinating seeds was in most instances much higher than in the surrounding soil, and these numbers were highest in the soils with the lowest stands, lowest in the soils with the highest stands, and intermediate in other soils. This zone, surrounding the germinating seed and developing under its influence a biological balance different from that of the soil farther removed, we designate the 'spermatosphere'. While there was a positive correlation between the numbers of *F. culmorum* isolates in the spermatosphere and the incidence of blight, the relation was not absolute for in this experiment *Acremonium* reduced the number of isolates in the soil but as in the check no seedlings survived. Also *Gliocladium* reduced the numbers of isolates to about 39% of the number in the check soil, but increased the stand to only 8%.

Microscopic examination of germinating seeds from sterilized soil infested with *F. culmorum* showed that hyphae of the pathogen grew abundantly on the hulls, on and in the endosperm, and attacked and killed the developing embryos. When *Phialophora* had been mixed with the infested soil the hyphae of this fungus were found growing in the hulls of the seeds while *F. culmorum* hyphae were less abundant on the hulls, scarce in the endosperms, and seldom were found in the embryos.

The above observation suggests that the hulls, which are the dead lemma and palea surrounding the caryopsis, provide a saprophytic substrate that may influence either the level of aggressiveness of the pathogen, the microbiological

balance between *F. culmorum* and other soil micro-organisms at that locus, or both of these. To test further these possibilities the hulls were removed from a quantity of seeds with forceps, and the stands resulting from sowing these dehulled seeds in soil infested with *F. culmorum* and antagonistic soil fungi were compared with the stands from normal seeds in the same soils. The results (Table VIII) showed that there were no significant differences in the stands from normal and dehulled seeds in uninfested sterilized and unsterilized soils, or in sterilized soil infested with *F. culmorum* alone. Nevertheless, the

TABLE VIII

THE EFFECT OF REMOVING THE HULLS OF BROME GRASS SEEDS ON THE STANDS IN VARIOUSLY INFESTED SOILS

Soil	Infestation	Stand, %	
		Normal seeds	Dehulled seeds
Sterilized	Check	92	90
"	<i>F. culmorum</i>	0	3
"	<i>F. culmorum</i> + <i>Phialophora</i>	45	24
"	<i>F. culmorum</i> + <i>Gliocladium</i>	7	22
Unsterilized	Check	83	86
"	<i>F. culmorum</i>	17	60

stand in infested soil to which *Phialophora* was added was lower from dehulled than from normal seeds. The reverse relation was shown for *Gliocladium* and unsterilized soil, for in these instances the removal of the hulls caused the stand to be significantly increased.

Evidence is thus presented that in the absence of effective competition *F. culmorum* may readily colonize the hulls and proceed to make a virulent attack on the germinating seed. However, in the presence of other soil micro-organisms the hulls may be colonized by some of these organisms as well as by *F. culmorum*. The hulls may therefore be decisive in determining the microbiological balance in this strategic locus and thereby influence the attack of the seedling by a pathogen present in the soil.

#### D. Variations in Microbiological Antagonism in Different Field Soils

During the summer of 1944, one sample of clay and 15 samples of loam soil differing in cultural history were collected from fields near St. Catharines and Vineland, Ont. Four 5 in. pots were filled with each soil, two of which were infested with 4% of *F. culmorum* inoculum, and one day later 25 seeds of crested wheat grass were sown in each pot. When no inoculum was added to these soils the stands of seedlings (Table IX) with three exceptions varied only from 70 to 78%; in a loam soil from a field of alfalfa and timothy and in loam from a field in which alfalfa had grown for a number of years followed by corn, it was as high as 84%, while in loam from grass sod the stand was only 64%. When these soils were uniformly infested with *F. culmorum*, the stands varied

TABLE IX

PERCENTAGE STAND FOLLOWING EQUAL INFESTATION WITH *F. culmorum* OF DIFFERENT FIELD SOILS COLLECTED NEAR VINELAND AND ST. CATHARINES, ONT.

Sample number	Soil texture	Cultural history	Uninfested soil	Infested soil
1	Clay	Alfalfa and timothy mixture	72	16
2	Loam	Alfalfa and timothy mixture	84	72
3	"	Oats after other cereals	78	36
4	"	Grass (in fence corner)	72	62
5	"	Grass (roadside)	76	76
6	"	Grass (pasture)	74	74
7	"	Grass (roadside)	72	66
8	"	Grass sod (black)	64	58
9	"	Grass sod (brown)	74	42
10	"	Wheat after other cereals	70	58
11	"	Corn after alfalfa	84	48
12	"	Old stand alfalfa and timothy (timothy killed)	74	40
13	"	Old stand alfalfa and timothy (alfalfa killed)	72	58
14	"	Peach orchard	78	56
15	"	Wooded area (under sparse growth of trees)	78	48
16	"	Wooded area (under dense growth of trees)	70	26

from 16 to 76%. The highest stands were in loam soils that had grown grass or an alfalfa-grass mixture for a considerable period of time. The lowest stands were in clay, also from a field growing alfalfa and grass, and in loam from a wooded area. Intermediate stands were generally obtained in soil from cultivated fields.

In October of the same year quantities of the first four soils listed in Table IX were collected for further experimentation. The clay and loam soils from the same field of alfalfa and timothy were selected because, while they had identical cultural histories, *F. culmorum* inoculum caused more seedling blight in the clay than in the loam. The other two soils were selected because one had grown cereal crops for a number of years, while the other had grown grass without being cultivated for many years. Hence these four soils gave contrasts in texture and cultural history. Fifty brome grass seeds were sown in each of four 6 in. pots of each soil infested with 4% *F. culmorum* inoculum by weight, and in two pots of each soil not infested. The stands were counted two weeks later. From two experiments using three Petri dishes for each dilution, counts were made of the numbers of bacteria, *F. culmorum* isolates, and other fungi per gram in the infested soil. The stands in uninfested and infested soils, and the numbers of soil fungi and bacteria as well as the numbers of *F. culmorum* isolates in the infested soils are given in Table X.

The stands in the uninfested soils did not vary more than 8% whereas, in the infested soils, it was again significantly lower in the clay than in loam from the same field. There was little variation in the stands among the loam soils of different cultural history. If seedling blight were a function simply of the development of *F. culmorum* in the soil, the numbers of isolates should be higher in the infested soils with the lower stands. The number of isolates of

TABLE X

RELATION BETWEEN SEEDLING STANDS AND THE NUMBERS OF BACTERIA, FUNGI, AND ISOLATES OF THE PATHOGEN IN FOUR VINELAND SOILS EQUALLY INFESTED WITH *F. culmorum*

Texture	Soil	Stand, %		Micro-organisms, thousands per gram		
	Cultural history	Uninfested	Infested	<i>F. culmorum</i> isolates	Other fungi	Bacteria
Clay	Alfalfa and timothy	75	22.5	123	300	17,200
Loam	Alfalfa and timothy	80	41.5	101	280	16,900
"	Oats	72	40.0	110	270	57,900
"	Grass	72	37.2	48	320	17,000

the pathogen was 18% higher in the clay than in the loam from the same field, hence in this instance the numbers were highest in the soil with the most seedling blight. The numbers in the three loam soils, however, varied greatly without relation to seedling stand. Similarly if suppression of blight in these soils were a function of the numbers of fungi and bacteria populating them, these organisms should have been lowest in the clay, but such was not the case. There was no evident correlation between differences in numbers of fungi or bacteria and differences in seedling blight.

Results of temperature experiments with these soils (13) showed that seedling blight was more severe at all temperatures in the clay than in the loam soils. There was also a correlation between the numbers of *F. culmorum* isolates and seedling blight at the higher (20° to 35° C.) but not at the lower soil temperatures (10° to 15° C.). In agreement with the results given in Table X there was no consistent relation between numbers of soil fungi or bacteria in the soils and seedling blight when infested with *F. culmorum*.

A similar comparison of the stands of grass seedlings in field soils infested with 6% *F. culmorum* was made with soils collected near Harrow, Ont., which varied in texture from sand to clay. All had grown cereal or forage crops for a number of years. The resulting stands two weeks after sowing are given in Table XI. In the uninfested soils the stands varied little, but among the infested soils they varied from 10 to 54.5%. The lowest stands were in clay, that in clay loam somewhat higher, those in loam still higher, while the highest stands of all were in sandy soils. The stands were low in all four infested sterilized soils with little variation among them. However, in contrast with these results, in the same soils unsterilized and infested, the stands were slightly higher in the clay than in the lighter textured soils when they were sterilized before infestation. This suggests that the differences between the stands in the unsterilized field soils uniformly infested with *F. culmorum* is due to the influence of texture on the microbiological balance and the effect of this on the pathogen.

The numbers of fungi, bacteria, and actinomycetes per gram in two of the clays, a loam and a sandy soil (Table XI : Nos. 1, 2, 6, and 8), were counted by

TABLE XI

PERCENTAGE STAND OF BROME GRASS IN HARROW SOILS UNIFORMLY  
INFESTED WITH *F. culmorum*

Sample number	Soil texture	Last crop	Unsterilized soil		Sterilized soil infested with 3% <i>F. culmorum</i>
			Uninfested	Infested with 6% <i>F. culmorum</i>	
1	Clay	Timothy	85	10.0	9.0
2	Clay	Wheat	86	11.5	4.0
3	Clay	Oats	88	13.5	—
4	Clay loam	Alfalfa	87	19.5	—
5	Loam	Oats	91	41.5	—
6	Black loam	Oats	90	46.5	3.5
7	Sand	Grass	91	54.0	—
8	Sand	Oat stubble	86	54.5	3.5

the dilution plate method to investigate any possible correlation that might exist between numbers of micro-organisms and suppression of seedling blight in these soils. In addition the numbers of these organisms in the rhizospheres of brome grass seedlings were investigated. For these counts dilutions were made of the soil apart from the roots and as well of the rhizosphere soil of 10 day old seedlings. Dilutions of 1 : 10,000 for fungus counts were plated with peptone-glucose agar, Czapek's agar modified by reducing the sucrose content to 1%, and brome grass seed extract agar. The seed extract was prepared by autoclaving 200 gm. of brome grass seeds in 1000 cc. of water for 15 min. at 15 lb. pressure, filtering the liquid, and restoring to the original volume with water. Portions (200 cc.) of this extract were transferred to 300 cc. Erlenmeyer flasks, to each of which were added 0.4 gm. of sucrose and 5 gm. of agar. The flasks were plugged with cotton and sterilized. To each flask of this medium, as to flasks containing similar quantities of peptone-glucose and Czapek's agars, 1 cc. of normal sulphuric acid was added after the media were melted in preparation for pouring in the Petri dishes containing 1 cc. of the required dilutions of soils. Dilutions of 1 : 100,000 were plated in Petri dishes with sodium albuminate and soil extract agars (3) for counts of bacteria. The actinomycete colonies that could be distinguished from the bacteria were also counted on these plates.

In Table XII are given the stands of seedlings in the four soils infested with 6% *F. culmorum* inoculum. The numbers of fungi, bacteria, and actinomycetes in the rhizosphere of seedlings as well as in the soil apart from the roots in pots of the same soils not infested with *F. culmorum* are also given. The numbers of fungi are the averages from three Petri dishes of each of the three acidified agars, and the numbers of bacteria and actinomycetes are the averages from three Petri dishes of albuminate and two of soil extract agar. The results show that the stands were lowest in the two clays, much higher in the loam, and highest of all in the sand. There was no consistent correlation between the numbers of actinomycetes in the soil and the stands of seedlings,

TABLE XII

THE NUMBERS OF MICRO-ORGANISMS IN THE RHIZOSPHERE AND IN THE SOIL APART FROM THE ROOTS OF SEEDLINGS 10 DAYS AFTER EMERGENCE IN DIFFERENT FIELD SOILS AS COMPARED WITH THE STANDS WHEN THE SOILS WERE INFESTED WITH 6% *F. culmorum* INOCULUM

Soil	Stand when infested, %	Numbers in thousands per gram					
		Rhizosphere			Soil apart from the roots		
		Fungi	Bacteria	Actino- mycetes	Fungi	Bacteria	Actino- mycetes
Clay (oats)	15	271	70,594	3511	205	37100	2125
Clay (timothy)	10	583	94,438	1532	240	39680	1625
Loam (oats)	43	346	120,746	3200	180	26354	2347
Sand (oats)	51	200	109,000	2531	149	21318	2439

whereas both bacteria and fungi were more abundant in the clays than in the loam or sand. This is contrary to what would be expected if the suppression of the pathogenicity of *F. culmorum* were a function of the numbers of these micro-organisms, for the stands were the lowest and blight most severe in the clay soils. In the rhizospheres of seedlings all three groups of micro-organisms, and especially the bacteria, were more abundant than in the surrounding soil. There was no consistent relation between the numbers of fungi or actinomycetes and stands, but bacteria were less abundant in the rhizospheres in the clay than in the loam and sandy soils.

Another experiment was carried out with the same soils, but this time, five days after sowing, the seedlings, which were just about to emerge, were removed from the soil, washed in sterile water, and further dilutions were made for plating. Counts were also made of the micro-organisms in the surrounding soil. Since the soils had been kept moist for considerably longer than for the preceding experiment, the results of the two experiments are not quantitatively comparable. The stands of seedlings in duplicate pots of these soils infested with *F. culmorum* are given in Table XIII along with the numbers of fungi, bacteria, and actinomycetes in the soil closely associated with the germinating seeds and in the surrounding soil.

As in the previous experiment the stands were lower in the two clay soils than in the loam and sandy soils equally infested with *F. culmorum*. The numbers of fungi were higher in the spermatosphere than in the surrounding soil, but, as was the case with the rhizospheres of 10-day-old seedlings there was no consistent relation between these numbers of fungi and the stands in the infested soils. The numbers of actinomycetes were actually lower in the soil associated with the germinating seeds than in the surrounding soil, and there was no consistent relation with stands. The numbers of bacteria, however, were from 233% to 830% higher in the spermatosphere than in the surrounding soil and were much higher in association with seeds germinating in sand and loam than in clay soils. Hence there is a correlation between



TABLE XIII

NUMBERS OF MICRO-ORGANISMS IN THE SOIL APART FROM AND ASSOCIATED WITH GERMINATING SEEDS FIVE DAYS AFTER SOWING IN DIFFERENT FIELD SOILS, AS COMPARED WITH THE STANDS WHEN THE SOILS WERE INFESTED WITH 6% *F. culmorum*

Soil	Stand when infested, %	Numbers in thousands per gram of soil					
		Spermatosphere			Apart from the seedlings		
		Fungi	Bacteria	Actino- mycetes	Fungi	Bacteria	Actino- mycetes
Clay (oats)	23	253	289,164	631	219	86,721	1559
Clay (timothy)	32	350	339,246	246	268	62,716	692
Loam (oats)	67	415	437,854	242	215	78,157	2806
Sand (oats)	74	253	424,124	744	121	45,583	1322

these greatly increased numbers of bacteria and the stands of seedlings obtained when these soils were infested with *F. culmorum*.

### Discussion

Most of the fungi that have been shown in these studies to be pathogenic to brome and crested wheat grass seedlings have also been reported as pathogens of cereal grasses in Canada (2). In recent years it has been shown repeatedly that many of the soil-borne pathogens of cereals are also destructive to forage grasses (4, 10, 11, 12, 15, 16). In addition it was shown in these studies that pathogenic *Helminthosporium* and *Fusarium* isolates were carried on brome and crested wheat grass seeds. The carrying of such pathogens on seeds is also an important consideration in seedling blight and root rots of cereals (1, 5, 6). Such close parallels in the seedling blight and root diseases of the cereal and forage grasses stress the need for considering, from a disease standpoint, the relations of forage grasses in crop rotations including cereals.

Although the cultivation of susceptible crops may have an important influence on the prevalence of seedling pathogens in the soil, other factors are also of major importance. The studies of other workers (7, 8, 9) have shown that soil micro-organisms influence the virulence of fungi that cause root rots of wheat. The studies reported here have considered the influence of soil micro-organisms on the virulence of *F. culmorum* to grass seedlings, and on its development in the soil.

The addition of 1% of *F. culmorum* inoculum was as effective as larger amounts in causing severe seedling blight in sterilized soil. In unsterilized soil, the incidence of blight increased as the amount of inoculum added to the soil was increased, but even 6% of inoculum caused less blight than did 1% in sterilized soil. If the concentration of the pathogen in the soil is decisive in determining disease incidence the above results can be explained on the basis of the rapid increase of the pathogen to a high concentration in sterilized soil as contrasted with the less striking increase in unsterilized soil. Hence,

within wide limits, the initial concentration of inoculum in sterilized soil is of little importance because of the rapidity with which it increases, whereas the initial concentration in unsterilized soil is important in determining the resulting incidence of blight because the increase is held in check by the other micro-organisms in the soil.

In sterilized soil the effect of temperature on blight appeared to be the result of its effect on the development of the pathogen in the soil. Further support for the view that seedling blight depends to a large extent on the concentration of the pathogen was obtained from experiments that indicated that with increased rates of infestation of sterilized soil, the effect of temperature on blight could be largely obscured and blight become equally severe at all temperatures. In unsterilized soil the relation of temperature to the development of *F. culmorum* was influenced by soil micro-organisms. These suppressed the pathogen most at the temperatures that were optimum in sterilized soil, so that its maximum development in the unsterilized soil was at significantly lower temperatures. The optimum temperature for blight was also lower in unsterilized than in sterilized soil. Again the evidence indicates that the incidence of blight is a function of the concentration of the pathogen in the soil. The effect of temperature and micro-organisms on blight is apparently the result of their influence on the development of *F. culmorum* in the soil.

The differences in the antagonistic effects of selected soil fungi and of unsterilized soil offered a suitable opportunity of comparing and contrasting their relative influences on *F. culmorum* development and on the incidence of blight. Such comparisons showed that there was no consistent correlation between their effect in suppressing the development of the pathogen in the soil and their effect in suppressing blight. However, the development of the pathogen in the environment in the immediate vicinity of the germinating seeds was different from that in the surrounding soil and did vary with disease incidence. This locus, in which the germinating seed induces a characteristic microbiological balance is designated as the 'spermatosphere'. Furthermore, in different field soils the biological balance in this spermatosphere was shown to be different although in all of them the numbers of bacteria in the spermatosphere were correlated with the suppression of blight, while the numbers in soil apart from the seedlings were not.

There are, therefore, two soil environments in which the microbiological balance has an important bearing on seedling blight. The microbiological balance in the general soil environment apart from the seedlings is of primary importance in that it must contain the pathogen in an effective concentration. The microbiological balance in the other environment in the immediate vicinity of the germinating seeds, the spermatosphere, depends on that of the surrounding soil but is very different from it. The resulting development in the spermatosphere is of major importance in determining the attack on the young seedling by a pathogen existing in the soil.

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# HEART ROT OF OAKS CAUSED BY *POLYPORUS OBTUSUS*<sup>1</sup>

By C. G. RILEY<sup>2</sup>

## Abstract

*Polyporus obtusus* Berk. is a heartwood decaying fungus that attacks several American species of broadleaved trees, principally oaks. It has a wide geographical range, but tends to occur in localized concentrations within which it causes great destruction, while elsewhere it is rarely found. On account of its peculiar distribution, *P. obtusus* is not well known, and the published references to it are comparatively few. A local concentration of this disease, the only one known in Canada, occurs at the Petawawa Forest Experiment Station, Ont. Here, oak trees from 0.8 to 4.8 in. in diameter were being attacked. The resulting white rot continues to advance into the sapwood, ultimately causing death of the tree. The large, cream-coloured, bracket-like sporophores are borne on the diseased trees in great abundance in some seasons, and are comparatively scarce in others.

*Polyporus obtusus* Berk. is perhaps the least known of all American polypores of comparable geographical range and destructive capacity. Published references are strikingly few. What appears to be the only original general account of the disease caused by this fungus, was published by Spaulding (8) in 1905. Von Schrenk and Spaulding (7) dealt with it again in 1909, and Lorenz and Christensen (2) devoted a brief paragraph to it in 1937. Other references are primarily taxonomic, or are simply records of occurrence. The species has been omitted frequently from systematic accounts of the polypores, even where these deal with regions within which it is known to occur. These circumstances seem to suggest that *P. obtusus* is an unimportant pathogen of rare occurrence; indeed, it is noted as "rare" by some authors. Actually, however, it is very widely distributed in North America, and occurs on a number of deciduous tree hosts, principally oaks. In a personal letter, dated February 16, 1946, the late Dr. L. O. Overholts of Pennsylvania State College kindly provided the following information for the purpose of this publication. In his collection are specimens of *P. obtusus* from Pennsylvania, New Jersey, Maryland, District of Columbia, Virginia, North Carolina, Georgia, Florida, Alabama, Louisiana, Mississippi, Tennessee, Wisconsin, Minnesota, Iowa, Missouri, Arkansas, Oklahoma, Kansas, Arizona, New Mexico, Oregon, and Ontario. The species has also been recorded at Berthierville, Que. (6). These records greatly enlarge the previously published geographical range of the fungus. Regarding hosts, Dr. Overholts stated that he had or knew of reliable records of the occurrence of *P. obtusus* on *Quercus*, *Acer*, *Juglans*, *Liquidambar*, *Fagus*, *Robinia*, *Pyrus*, and *Carya*. Add to the foregoing the published facts that "the disease . . . . is quite destructive locally to several species of oak trees" (8), and "a large number of trees are usually found affected in a locality" (7), and the situation becomes somewhat

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of a puzzle. Actually, it appears that the fungus occurs in dense concentrations in widely separated localities within which it is a vigorous and destructive pathogen, but outside of which it is of comparatively rare occurrence.

The Ontario record mentioned above refers to a dense, local concentration of *P. obtusus* at the Petawawa Forest Experiment Station, about 95 direct miles northwest of Ottawa. Here the principal host is red oak (*Quercus borealis* Michx.), though white oak (*Q. alba* L.) and bur oak (*Q. macrocarpa* Michx.) are also attacked. Most of the observed infected trees are on poor sites for these species, principally on rocky ridges, and associated with aspen, jack pine, and red maple.

A 1/10 ac. sample plot was examined in a locality where sporophores of *P. obtusus* were particularly abundant. The plot contained 61 red, and 12 white oaks, many of which occurred as coppice clusters. They ranged in age from 15 to 25 years, were 0.8 to 4.8 in. in diameter at breast height, and 8 to 29 ft. high. Heartwood decay, typical of that caused by *P. obtusus*, was found in 57 of the red, and in 3 of the white oaks. The remaining 13 trees were all among the youngest and smallest of those examined. Sporophores of the fungus were associated with the decay in 11 of the red oaks and all three of the infected white oaks. Some sporophore-bearing bur oaks were found nearby.

The nature of the disease and the appearance of the affected wood on the Petawawa plot were essentially as described in the references already cited. The fungus appeared to have gained entrance through various kinds of wounds such as frost cracks, mechanical abrasions, and branch snags. It is of interest to note that Spaulding (8) found the disease consistently associated with insect tunnels. Some badly infected trees exhibited no external symptoms or wounds to suggest that they were diseased. In some trees, the decay occurred only in the lower trunk, and in others, only in the crown.

In its early stage, the decay appears as creamy-white concentric lines in cross section, or streaks in longitudinal section, within the early wood of the annual rings (Fig. 5). Badly decayed wood is spongy, and creamy white when freshly exposed, tending toward tan on drying. In cross-section, a

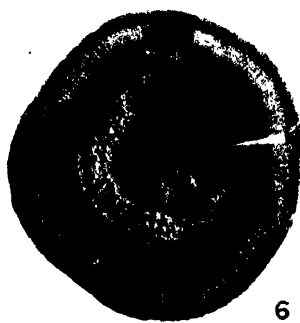
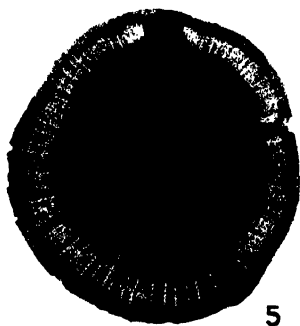
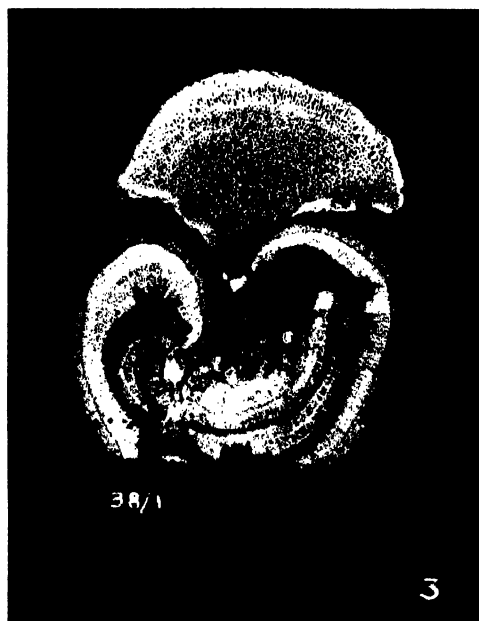
FIG. 1. Sporophore of *Polyporus obtusus* growing from an open knot on living red oak. (Diameter of host at top of section, 3 in.)

FIG. 2. Longitudinal section through specimen shown in Fig. 1. Note the structure of the sporophore, and the advanced decay surrounded by dark-stained heartwood.

FIG. 3. Sporophore of *Polyporus obtusus* growing from an old wound in living white oak. (Half natural size.)

FIG. 4. The ultimate fate of the host. A living red oak about 4.5 in. in diameter, badly decayed by *Polyporus obtusus*, and broken at the weakened point.

FIGS. 5, 6, and 7. *Polyporus obtusus* decay in a living red oak main stem at a height of between 4 and 5 ft. (diameter 2.4 in.). Fig. 5 shows the condition near the upper extremity of the advanced decay. Note the decay confined to the early wood of the annual rings. The remaining heartwood is darkly stained. Fig. 6 shows the condition at a point 2.5 in. below that shown in Fig. 5, while Fig. 7 is from 5.5 in. lower than Fig. 6. Note in Fig. 7 the sharply defined margin of the densely stained wood in contact with the healthy sapwood.





core of advanced decay may be delimited by a narrow dark line, or the entire remaining heartwood region may be abnormally dark (Fig. 6). Frequently, this discoloration becomes more intense toward the periphery. It may terminate abruptly in a dark line adjacent to the sapwood (Fig. 7). Longitudinally, the region of advanced decay may terminate rather abruptly (Figs. 5 to 7), or it may continue for a considerable distance, tapering to thin central or excentric streaks that sometimes extend into the branches. Typically, the dark-stained wood that surrounds the rot extends longitudinally beyond the decay region as a central core far up the stem and into the branches. Numerous tests were made by means of agar cultures, to determine whether *P. obtusus* was present in this dark-stained wood. The results were all either negative or inconclusive. Several organisms developed in the cultures, but *P. obtusus* was not found. Also, this fungus could not be obtained in culture from the dark-stained wood laterally surrounding the advanced decay, although it could be isolated readily from the margin of the decayed region. The cultural characteristics of the fungus have been described by Davidson, Campbell, and Vaughn (1). As the fungus advances within the infected tree, the decayed core continues to enlarge until the adjacent sapwood region is entirely involved and the affected branch or stem either dies or breaks over at the weakened point (Fig. 4).

Sporophores commonly occur at branch snags or open knots (Figs. 1, 2), at wounds (Fig. 3), and at the point of breakage (Fig. 4). They have been described in detail by Lowe (3), Overholts (4, 5), and others. They are conspicuous in the woods by reason of their size and colour (Fig. 4), being commonly 2.5 to 5.0 in. broad and 1.5 to 2.5 in. thick. They are firm, strongly convex, and bracket-like. The velvety to coarsely tomentose upper surface is creamy white to grayish, while the pore surface tends toward a darker cream or tan. The whole may become dark tan on drying. The tubes are coarse, with irregular mouths, and are commonly 0.5 to 1.0 in. long. The annual sporophores may occur in great abundance in one season, and be quite scarce in the same locality another year.

It is stated by Lorenz and Christensen (2) that *P. obtusus* is responsible for considerable losses in oak in the Central States. In Ontario, only the three species of oak already mentioned extend as far north as the Petawawa region, and there they do not form a commercially important constituent of the forest. Red oak is cut for sawlogs on the better sites, and here it is characteristically sound. Although no data are available to indicate the amount of loss caused by *P. obtusus*, the disease is not believed to be of great economic importance in Ontario at present. Further investigation in this respect is required. In the light of present knowledge, the most interesting feature of the disease lies in the peculiarity of its local and geographical distribution.

### Acknowledgments

The assistance of Dr. Ruth Macrae and Dr. Mildred Nobles in verifying identifications of sporophores and cultures is gratefully acknowledged.



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## L'ANTAGONISME DE CERTAINS MICRO-ORGANISMES ENVERS *CORYNEBACTERIUM SEPEDONICUM*<sup>1</sup>

PAR CHAMPLAIN PERRAULT<sup>2</sup>

### Sommaire

La croissance de *Corynebacterium sepedonicum* (Spieck. & Kott.) Skaptason et Burkholder sur milieu gélosé est entravée par un bon nombre de micro-organismes isolés de tubercules de pommes de terre en putréfaction. Parmi ceux-ci on a observé l'influence antibiotique de dix cultures bactériennes différentes, quatre *Actinomyces* sp., quatre *Penicillium* sp. et un *Chaetomium* sp. Une culture bactérienne et un *Actinomyces* sp. effectuèrent une lyse de *C. sepedonicum*, tandis que trois autres micro-organismes favorisèrent sa croissance.

### Introduction

Au cours des études que l'on a poursuivies aux fins de déterminer l'importance des nombreux organismes associés à *Corynebacterium sepedonicum* dans les tubercules de pommes de terre atteints de pourriture, un certain nombre d'entre eux semblaient exercer un antagonisme envers l'agent pathogène de la pourriture du cerne, communément désigné sous le nom de flétrissure bactérienne.

Burkholder (2), en 1938, a rapporté que les colonies de *C. sepedonicum* sur gélose se développent plus rapidement autour de certains agents contaminants. Snieszko et Bonde (3), en 1943, ont attribué une certaine influence toxique aux organismes secondaires observés en culture parmi les colonies de *C. sepedonicum*. Brown et Boyle (1), en 1944, ont obtenu des indications à l'effet que cet agent pathogène est sensible à l'action de la pénicilline.

Parmi les antagonistes que l'on a isolés, les bactéries prédominent. Il y a aussi quelques *Actinomyces*, des *Penicillium* et autres champignons que l'on désigne temporairement par leurs numéros de culture en attendant de les identifier et de déterminer leur importance dans leur association avec *C. sepedonicum*.

### Partie Expérimentale

#### Technique des Essais

Les cultures, à l'exception de *C. sepedonicum*, sont maintenues sur milieu gélosé ordinaire à base de pommes de terre additionné de 15 g. de dextrose,

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tandis que *C. sepedonicum* est cultivé sur le même milieu auquel on a ajouté 1 g. d'extrait de levure Bacto et 1 g. de phosphate dibasique de sodium.

Des tubes contenant 15 ml. de ce milieu à l'état liquide ont été ensemencés avec *C. sepedonicum* en suspension dans de l'eau stérilisée. Les tubes ainsi ensemencés ont été versés dans des boîtes de Petri de 90 mm. de diamètre que l'on a conservées à la température du laboratoire. Après une journée d'incubation, les boîtes ont été ensemencées au centre, à l'aide d'une aiguille, avec un de chaque organisme à l'essai. Les cultures qui ont servi d'inoculum étaient âgées de dix jours, tandis que celles de *C. sepedonicum* qui ont servi à la préparation de la suspension utilisée dans l'ensemencement des boîtes de Petri étaient âgées de douze jours.

### Résultats

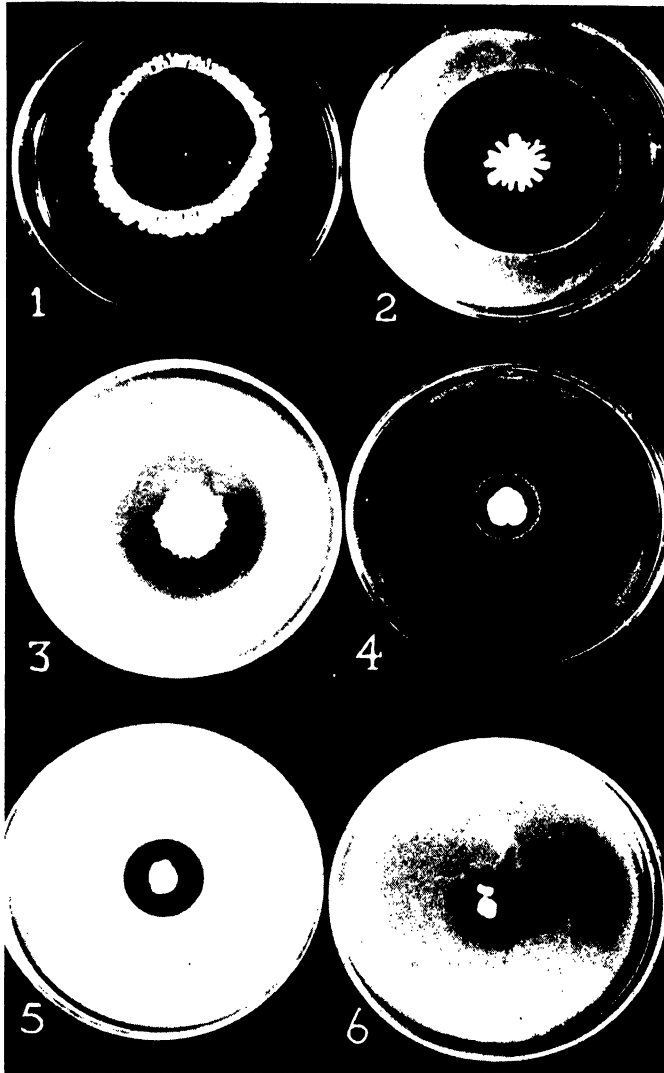
Une des bactéries, le numéro 200, s'est développée rapidement et a couvert la surface de l'agar, empêchant par le fait même toute croissance de *C. sepedonicum*. Neuf autres bactéries se sont montrées antagonistes à *C. sepedonicum*, tandis qu'une autre, numéro 196, a semblé quelque peu favoriser sa croissance.

Lors de son isolation dans la boîte de Petri, où il poussa accidentellement, le numéro 200 a produit une lyse de *C. sepedonicum*. La culture 239, grâce à son expansion et à la diffusion rapide de sa substance antibiotique, a empêché toute croissance de *C. sepedonicum* dans la boîte de Petri (Fig. 1).

La substance produite par la culture 124 s'est diffusée rapidement dans le milieu gélosé et a stimulé quelque peu la croissance de *C. sepedonicum* à la marge extérieure de la zone hyaline (Fig. 2). Ce phénomène est plus accentué avec *Bacillus subtilis* qui a produit aussi une substance antibiotique (Fig. 3). Certaines bactéries, entre autres les numéros 118, 188, 223 et 241, ont également démontré un puissant antagonisme envers *C. sepedonicum*. Quelques-unes, malgré leur expansion excessivement lente et limitée sur le milieu gélosé, ont exercé une influence antibiotique aussi prononcée que d'autres dont la croissance a été plus rapide et abondante.

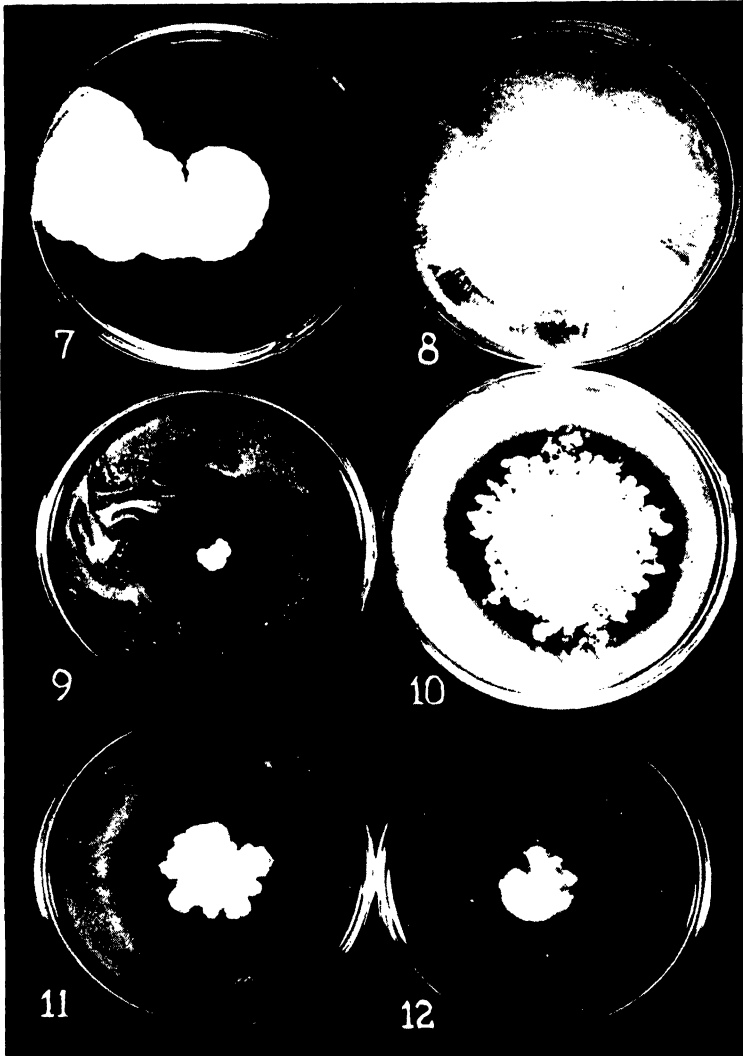
Au nombre des *Actinomyces* qui ont produit des substances antibiotiques mentionnons *A. scabies* et les espèces désignées par les numéros 189, 235 et 238. *A. scabies* a produit une zone hyaline bien définie autour de laquelle il y a eu, comme pour *Bacillus subtilis* et autres micro-organismes, croissance abondante de *C. sepedonicum*, conséquence d'une stimulation attribuée à une faible concentration de la substance antibiotique. Au delà de cette zone de croissance intensive l'on a observé une autre sphère de cinq à six mm. de largeur, probablement due à l'inégalité du fond de la boîte de Petri (Fig. 4). Les *Actinomyces* sp. 189 et 238 ont exercé une action antibiotique semblable à *A. scabies*. Cependant, la stimulation que l'on observe aux confins de la zone hyaline formée par ce dernier n'est pas aussi évidente avec *Actinomyces* 238 et nulle avec *Actinomyces* 189 (Fig. 5 et 6). *Actinomyces* sp. 235, en plus d'avoir produit la substance antibiotique la plus puissante des quatre organismes du même genre, a exercé une action lytique sur *C. sepedonicum*. On

# PLANCHE I



*Influence antibiotique des micro-organismes sur Corynebacterium sepedonicum.*

FIG. 1. Bactérie 239 n'a permis aucune croissance de l'agent pathogène. FIG. 2. Bactérie 124. FIG. 3. *Bacillus subtilis*. FIG. 4. *Actinomyces scabies*. FIG. 5. *Actinomyces* sp. 238. FIG. 6. *Actinomyces* sp. 189. A noter dans les figures 2, 3, 4, la croissance stimulée à la marge extérieure de la zone inhibitrice.



*Influence antibiotique des micro-organismes sur Corynebacterium sepedonicum.*

FIG. 7. *Penicillium* sp. 234. Une faible ligne de croissance est apparente à la partie supérieure de la boîte de Petri. FIG. 8. *Penicillium notatum*. FIG. 9. *Actinomyces* sp. 235 effectue la lyse de *C. sepedonicum*. FIG. 10. *Chaetomium* sp. 48. FIG. 11. Bactérie 236. FIG. 12. Bactérie 180.

peut voir l'effet de cette lyse en observant de près la figure 9. On y distingue une étroite zone intermédiaire où se produit la dissolution de *C. sepedonicum* entre la zone de croissance de cet organisme et la zone hyaline. *Actinomyces* sp. 34 n'a exercé aucun effet antibiotique, mais au contraire a favorisé la croissance de l'agent pathogène.

Quatre différentes cultures de *Penicillium* ont manifesté, à divers degrés, leur antagonisme envers *C. sepedonicum*. Cependant, le *Penicillium* sp. 234 se révéla le plus antagoniste des quatre et la substance antibiotique qu'il secréta se diffusa très rapidement dans le milieu pour enrayer, presque totalement, la croissance de *C. sepedonicum* (Fig. 7). La lignée de *P. notatum* utilisée n'a eu aucun effet sur l'agent pathogène de la pourriture du cerne dont les colonies se sont développées en abondance sous la croissance mycélienne du champignon. Celui-ci a couvert presque toute la surface du milieu gélosé (Fig. 8). Il est vrai que *P. notatum* avait déjà été repiqué plusieurs fois depuis que nous l'avions en laboratoire, mais *Penicillium* sp. 234, ainsi que les autres, l'avaient été aussi souvent depuis leur isolation.

Parmi les autres champignons que l'on a isolés et qui ont montré un certain antagonisme envers *C. sepedonicum* il y a *Chaetomium* sp. 48 dont la substance antibiotique se révéla aussi puissante que celle de certaines bactéries (Fig. 10).

### Discussion et Conclusions

Il est évident qu'un bon nombre d'organismes, isolés de tubercules de pommes de terre atteints de pourriture, sont antagonistes à *Corynebacterium sepedonicum* et que la croissance lente de celui-ci sur gélose favorise l'action antibiotique des premiers. On n'a jamais pu isoler *C. sepedonicum* d'un sol non-stérilisé, même quelques jours après l'y avoir enfoui. Il est probable que la présence de ces micro-organismes et d'une multitude d'autres dans le sol entravent la croissance de *C. sepedonicum* ou produisent une lyse de celui-ci. C'est probablement la principale cause des échecs subis lors des essais d'isolation de cet organisme. Il m'est arrivé quelques fois de ne pouvoir isoler *C. sepedonicum* de tubercules de pommes de terre malades, à cause de la présence de certains organismes secondaires qui devaient sans doute en empêcher la croissance dans les boîtes de Petri.

Il serait intéressant de connaître l'action de chacun de ces organismes envers *C. sepedonicum* dans un sol stérilisé, mais il faudrait au préalable, avoir l'assurance que cet agent pathogène puisse s'y développer ou au moins y vivre durant quelques jours. A ce sujet les résultats négatifs obtenus à la suite d'un essai préliminaire ne concordent pas avec ceux de Snieszko et Bonde (3).

Dans les essais rapportés ici-même, l'influence antibiotique des divers organismes a pu être observée dès la cinquième journée après l'ensemencement, alors que les colonies de la plupart des antagonistes étaient encore petites. Cependant, la dimension des zones d'inhibition est restée sensiblement la même, tandis que celle des colonies antagonistes a continué d'accroître



pour atteindre des proportions que l'on peut observer dans les figures 1 à 12. Les substances antibiotiques produites par les antagonistes n'ont pas empêché la croissance de la plupart de ces micro-organismes.

### Summary

The growth of *C. sepedonicum* in agar media was impeded by several micro-organisms isolated from rotted potato tubers affected with ring rot. Some of these organisms produced antibiotic substances that diffused quite readily through the medium and prevented all growth of the pathogen. Among these organisms there were 10 different bacteria, four *Actinomyces* sp., four *Penicillium* sp., and one *Chaetomium* sp. One bacterial culture and one *Actinomyces* sp. produced a lysis of *C. sepedonicum*, while three other organisms seemed to stimulate its growth.

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## STUDIES ON STEAM STERILIZATION OF SOILS

### I. SOME EFFECTS ON PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES<sup>1</sup>

BY S. N. MALOWANY<sup>2</sup> AND J. D. NEWTON<sup>3</sup>

#### Abstract

Effects of steam sterilization and recontamination with original soil, on certain physical, chemical, and biological properties of four Alberta soils were investigated. The soils were Edmonton black loam, Vegreville black fine sandy loam, Gros Ventre brown loam, and Fallis gray silt loam. Certain physical properties were affected by steam sterilization as follows: (1) capillary rise of moisture was greatly retarded in all four soils following sterilization; (2) the percentages of the different separates as determined by ordinary mechanical analysis were not changed significantly by sterilization; (3) water-holding capacity and moisture content at the 'sticky point' were slightly reduced, and, in three of the soils, shrinkage was slightly increased by sterilization, but these effects were of uncertain significance; (4) the pH values of these soils were not changed appreciably by sterilization. The following chemical changes were produced by steam sterilization: (1) water-soluble phosphate was generally greatly increased and water soluble sulphate somewhat increased by sterilization. Easily soluble phosphorus (soluble at pH 3) was increased by about one-third in the two black soils rich in organic matter, but not in the other soils; (2) ammonia accumulated rapidly in the sterilized recontaminated black and brown soils for four to six weeks, and then returned to normal in 8 to 12 weeks. Ammonia accumulated less rapidly and to a smaller degree in the gray soil; (3) nitrification was suppressed for about 6 to 10 weeks in the sterilized recontaminated soils, but was later more active in these soils than in the unsterilized (especially in the black soils rich in organic matter).

#### Introduction

The study of some physical, chemical, and biological changes brought about by steam sterilization of soils, as reported in this paper, is part of a broader study of the inter-relationships of soil micro-organisms and plant diseases, and of soil micro-organisms and soil fertility. The study of these complicated inter-relationships may be somewhat simplified by first studying the relationships of a few known organisms on sterilized soil devoid of its enormous population. However, the study of effects of known micro-organisms in sterilized soil necessitates a study of effects produced by sterilization, because the sterilized soil may be quite different from the original soil, physically and chemically, and the results obtained may be misleading if the changes due to sterilization are not measured.

In practice, mainly in greenhouses, soils are partially sterilized to get rid of weeds, insects, and other undesirable or disease producing organisms. Complete sterilization is neither strived for nor desirable since there are many organisms in the soil that are beneficial to plant growth because they are

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responsible for the decomposition of organic matter and the elaboration of plant nutrients.

Literature dealing with the changes effected when soils are completely sterilized by steam is less abundant than that dealing with partial sterilization. Effects of various heat and chemical treatments in partially sterilized soils have been studied by many workers in England and the United States, and their findings would apply, in varying degrees, to the Alberta soils used in these investigations. However, different soils respond differently to such treatments, and, furthermore, the Alberta soils were sterilized completely rather than only partially.

Darbishire and Russell (7) noted that in steam sterilized soils heated to 120° C. the absorption of oxygen was greatly diminished, but that in partially sterilized soils, heated to 95° C., the absorption was considerably increased. They found that the crop could get from heated soils three times as much nitrogen and twice as much phosphorus and potash as from the unheated soils. They believed that the increased availability of the plant nutrients was connected with the modification of microbial flora, although they recognized that when soils were heated or sterilized by steam, a certain amount of decomposition took place.

Russell and Hutchinson (16) found that heating the soil by steam to 125° C. killed all the organisms present, and caused a production of 5 p.p.m. of ammonia, but after that first production no further change took place. Heating by steam to 98° C., or treating with toluene, increased ammonification greatly for a time, and they concluded that these treatments destroy organisms that are inimical to ammonifying bacteria. The number of bacteria increased greatly following partial sterilization, and this they attributed to the absence of protozoa. They found that heating to 98° C. not only destroyed the nitrifiers, but made the soil temporarily unfit for their development.

Lyon and Bizzell (11) observed that steamed soil, on standing up to three months without plants, steadily decreased in water soluble organic matter, ammonia, and other soluble nitrogenous matter. They found that nitrates remained low, up to 14 weeks in steamed and recontaminated soils, but that ammonia increased. Baldwin (1) reports a consistent increase in acid soluble phosphorus after sterilization. Pickering (14) and Newhall (13) found a lower water-holding capacity in steam sterilized soil.

Practical sterilization is discussed by Bewley (2), Senner (18), and Newhall (13). Newhall concludes that steam sterilization leaves no toxic residues or water-logging and leaves the soil in good tilth.

## Methods

The investigation of changes effected by steam sterilization of Alberta soils and subsequent microbial activities following recontamination was divided into two parts: first, the physical and second the chemical-biological changes. Four Alberta soils, Edmonton black loam, Vegreville black fine sandy loam,

Gros Ventre brown loam, and Fallis gray silt loam, were studied. The first two are high in organic matter and the last, very low.

In the study of the physical properties of these soils, the non-sterilized were compared with the sterilized with respect to mechanical analysis, water-holding capacity, shrinkage, sticky point, and capillary power. Since soil moisture relations affect greatly the ultimate productive power of the soil, certain differences in sterilized soils may be due to purely physical factors.

The chemical-biological studies included determinations of acidity, ammonia, nitrates, sulphates, easily soluble phosphorus, and ammonia production by pure cultures.

In the study of physical and biochemical properties, the non-sterilized soils were compared with the sterilized, and with the sterilized-recontaminated also, in the case of the biochemical properties.

For the study of physical properties the soils were sterilized in 1-gal. crocks, and for the study of biochemical properties the soils were placed in Erlenmeyer flasks and incubation was carried on in a dark control chamber at room temperature (23° C. to 27° C.).

Two nitrification experiments were carried out, the first for a period of 12 weeks and the second for 39 weeks. Nitrates were determined frequently throughout these periods, and one flask or tumbler was taken for each determination. Similar plans were followed in the case of the ammonification and sulphofication experiments. Easily soluble phosphorus was determined on the same sample as ammonia. The experiment on ammonia production by pure cultures of *Ophiobolus* and two other fungi, one antagonistic and the other not antagonistic to *Ophiobolus*, was carried out in Erlenmeyer flasks of sterilized Edmonton black soil inoculated with these organisms. The results of these experiments with pure cultures will be published in a subsequent paper together with results of other similar experiments.

Representative samples of the surface six inches of the four soils were obtained, air-dried, put through a 2 mm. sieve, and well mixed. The soils were brought to optimum moisture and allowed to stand for two days or less before sterilizing. Table I shows the optimum moisture used for each soil and a few other physical characteristics of the four soils.

Sterilization was done by steaming the soil at 15 to 17½ lb. pressure (approximately 120° C.) for 45 min. and again next day at the same pressure for 30 min.

After sterilization the soils were cooled and half of the sterile flasks were recontaminated for nitrification, ammonification, and sulphofication experiments.

In all cases where the soil was incubated, the flasks were taken out of the chamber every week or 10 days and brought up to optimum moisture with sterile water. By means of a stirring rod that had been placed in each flask through the cotton plug, the soils were cultivated after being watered.

TABLE I

## PHYSICAL CHARACTERS OF THE FOUR ALBERTA SOILS

—	Treatment	Hygroscopic moisture, %	Moisture-holding capacity, %	Optimum moisture, %	Real specific gravity	Sticky point moisture, %
Edmonton black park (loam to silt loam)	Non-sterilized		76		2.34	60
	Sterilized	5.96	72	36	2.40	58
Vegreville black park (sandy loam to fine sandy loam)	Non-sterilized		71		2.51	52
	Sterilized	4.52	71	30	2.54	48
Gros Ventre brown prairie (loam)	Non-sterilized		75		2.32	50
	Sterilized	2.13	74	32	2.33	46
Fallis gray wooded (silt loam)	Non-sterilized		59.5		2.54	33
	Sterilized	1.22	58.5	19	2.51	33

For mechanical analysis, Bouyoucos' hydrometer method (3) was adopted. Moist samples were used rather than dry ones because it was thought that following sterilization drying might affect the soil colloidal content and thereby alter changes induced by steam sterilization. The non-sterilized samples had the same amount of moisture added as the sterilized ones and were kept moist for the same length of time prior to mechanical analysis. Since it is more difficult to weigh accurately moist samples, owing to loss of moisture, a greater analytical error had to be contended with. Otherwise, in the main, the procedure recommended by Bouyoucos was followed. The samples were dispersed by adding water, treating with solutions of sodium oxalate and sodium silicate, and mixing in a mechanical stirrer.

Some seven determinations were made also by the pipette method for comparison with the hydrometer method.

The real specific gravity of these soils (specific gravity of soil particles) was calculated from water displaced in a volumetric flask by 10 gm. of oven-dry soil.

Water-holding capacity was found by the funnel method, using 50 gm. of soil. Readings were taken when drainage stopped, and in certain determinations readings were made at 15 min., one hour, and two hours.

The 'sticky point' moisture content of the sterilized and non-sterilized soils was determined by a somewhat arbitrary method. Air-dry 50 gm. samples were moistened with a measured amount of water and the moisture was worked into the soil with a spatula on a glass plate until the sticky point was nearly reached. This was judged by the appearance of wetness. Then the wet soil was kneaded by hand and a little more moisture worked into it, until the point of maximum stickiness was attained as judged by the adhesion to the palm of the hand and the force required to tear away the soil cake after it was squeezed in the hands.

Shrinkage was determined by a modification of Haines' mercury displacement method (8). The soils were brought to optimum moisture content, and half of each was sterilized as previously described. Then both the non-sterilized and the sterilized soils were air-dried and passed through a 100 mesh sieve. Into 50 gm. of soil on a glass plate a measured amount of water was incorporated by means of a spatula, until the sticky point was nearly reached. It was kneaded, shaped by hand, and moistened, more especially at the ends, to prevent cracking. The wet cylinder was weighed and its volume determined by placing it in a large glass test tube, which was then filled with mercury, and levelled off with a microscope slide. The buoyant effect was overcome by placing a perforated rubber stopper below the surface of the mercury. Weight and volume of the soil cylinder were measured at intervals of drying, first at room temperature, later in an oven at 100° C., and finally after drying for two days at 110° C. All data were reduced to the basis of 1 cc. oven-dry soil. Some difficulty arises in finding the first volume if the soil cylinder is too wet, as the buoyant force of the mercury compresses the soil and distorts the soil cylinder; also, small bubbles often adhere. But with a little drying these difficulties can be overcome and the volume measured accurately.

Capillary rise of water in the sterilized and unsterilized soils was determined by measuring the rise in glass tubes about 1½ in. in diameter. The soils were prepared, sterilized, and air-dried as previously described. The air-dried soil was then put through a 2 mm. sieve, and the tubes, wrapped at the lower end with cheesecloth, were filled uniformly with the soils. The tubes were set vertically in a pan to which water was added and maintained at a constant level. From the time of addition of water, measurements of capillary rise above the water level in the pan were taken at 5 min., 15 min., 1 hr., 2, 3, 4, 12, and 24 hr. and thereafter every day for 42 days, and at longer intervals up to 61 days from the beginning.

The pH values were determined by the quinhydrone electrode method. One day after sterilization, moist samples of sterilized and unsterilized soil equivalent to 5 gm. of water-free soil were diluted with 20 cc. of distilled water and pH values read at 1, 5, 15, and 30 min.

Water soluble phosphorus was determined colorimetrically on 1 : 5 water extract of soil, by the Parker and Fudge method (15). Moist samples equivalent to 50 gm. water-free soil were shaken for one hour and filtered through Berkefeld filters. Then 25 or 50 cc. portions of filtrate were evaporated with a little magnesium nitrate and ignited to remove organic matter before determining phosphorus. However, in a few cases, for purposes of comparison, the dried filtrates were not ignited.

Easily soluble phosphorus (soluble at pH 3) was determined colorimetrically by Truog's (19) method. Moist samples of soil equivalent to 1 gm. water-free soil were extracted by shaking for one hour with 200 cc. buffered pH 3.0 sulphuric acid solution, adjusted when necessary by the addition of one to seven drops of normal sulphuric acid. The pH values were determined with

the potentiometer before and after extraction. A few soil extracts, after being filtered through paper, were highly coloured and such extracts were cleared by means of activated charcoal. Blue colour was developed with ammonium molybdate-sulphuric acid solution and stannous chloride.

Nitrates were determined colorimetrically on 1 : 5 water extracts of soil by the phenol-disulphonic method as modified by Harper (9).

Ammonia was determined by the McLean and Robinson (12) method of leaching 25 gm. of soil with 500 cc. normal sodium chloride solution, distilling the soil extract with magnesium oxide, and collecting the ammonia in standard acid.

Water soluble sulphates were determined gravimetrically. The 1 : 5 extracts were filtered through Berkefeld filters, evaporated with magnesium nitrate, and ignited to destroy organic matter prior to solution in dilute hydrochloric acid and precipitation as barium sulphate.

## Results and Discussion

### MECHANICAL ANALYSIS

Mechanical analysis of 25 samples of soil were made and the results of 16 are represented in Fig. 1. Taking the silt particles limits as 0.002 mm. to 0.05 mm. in diameter, the Edmonton black park soil should be classed as loam to silt loam, the Vegreville black park soil as sandy loam (probably fine sandy loam), the Gros Ventre brown prairie soil as loam, and the Fallis gray wooded soil as silt loam. Differences between the sterilized and unsterilized soils were not obtained by the methods of mechanical analysis used in these experiments.

### WATER-HOLDING CAPACITY

The funnel method of determining water-holding capacity was used and the results indicate that the sterilized soils have about 1% lower water-holding capacity than the unsterilized, but the differences were not obtained in every case and cannot be taken as definitely significant. The water-holding capacities are shown in Table I. Pickering (14) and Newhall (13) report that sterilized soils have a lower water-holding capacity than unsterilized, but that the former hold water for a longer time.

### SHRINKAGE

The results of the shrinkage experiments are represented by Figs. 2 and 3. They show that during the early stages of drying there was a decrease in volume proportional to the amount of water lost. This decrease, represented graphically, is shown by the slant of the curve at an angle of approximately 45° to the abscissa.

There was a sudden change in the direction of the curves in Fallis gray wooded soil from about 0.34 cc. moisture per gram water-free soil up to the last stage of drying; the curves become almost parallel to the base (abscissa).

This stage of drying is accompanied by a loss in moisture that is relatively large in comparison with the loss in volume, and is called 'residual' shrinkage.

### PARTICLE SIZE SCALE

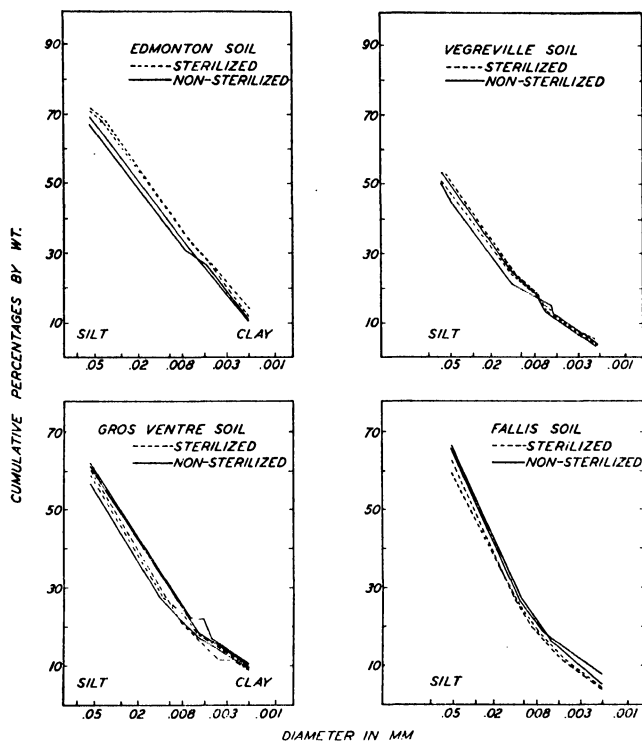


FIG. 1. Mechanical analysis of non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil, and Fallis gray wooded soil.

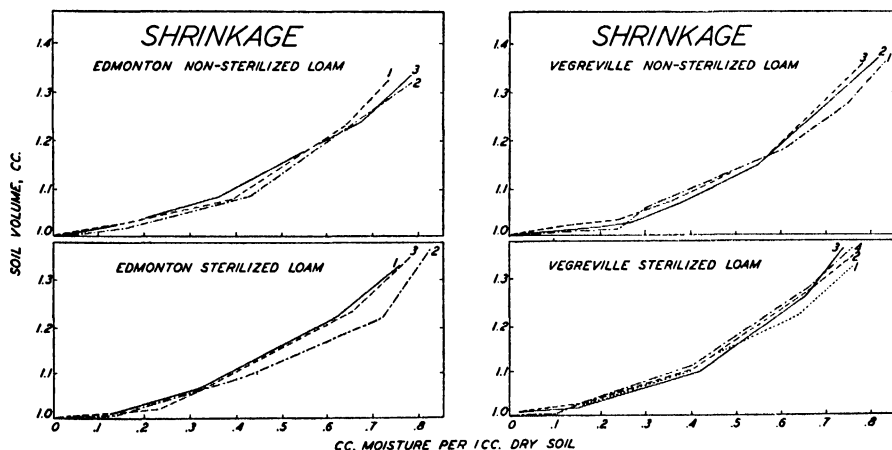


FIG. 2. Shrinkage of non-sterilized and steam sterilized Edmonton and Vegreville black park soils.



This residual shrinkage in the unsterilized Fallis soil amounted to approximately 0.02 cc. decrease in volume for 0.35 cc. of moisture lost, and, in the sterilized, 0.025 cc. for 0.33 cc. moisture lost.

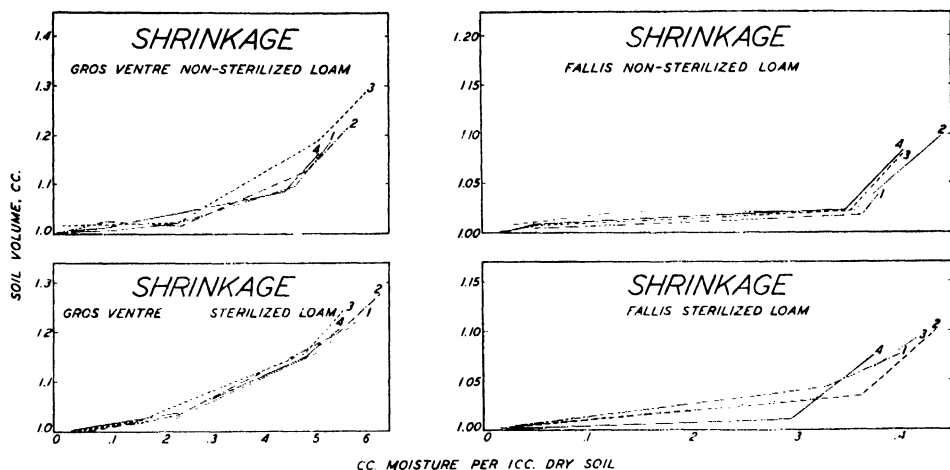


FIG. 3. Shrinkage of non-sterilized and steam sterilized Gros Ventre brown prairie soil and Fallis gray wooded soil.

The break between the 'normal shrinkage' and the 'residual' shrinkage is not so sharp in the Gros Ventre brown prairie soil as might have appeared if a determination had been made at about 0.37 cc. moisture content per cc. of dry soil. The volume of soil at 0.24 cc. of moisture, which is well within the region of 'residual' shrinkage, is, in the unsterilized, approximately 1.02 cc. while in the sterilized at the same moisture it is approximately 1.03 to 1.04 cc. Again this is a small difference, but the shrinkage is greater in the sterilized.

The Vegreville soil shows a gradual change instead of a sharp break. This is indicated by the points plotted between 0.1 cc. and 0.65 cc. of moisture. A slightly greater shrinkage in the case of the sterilized as compared with the unsterilized soil, of about the same magnitude as in the former two soils, was obtained below a moisture content of approximately 0.25 cc. The Edmonton soil shows the same trends as the Vegreville soil.

From Figs. 2 and 3 it appears that 'residual' shrinkage is greatest in Edmonton black soil, followed by Vegreville black, and least in Fallis gray wooded. This indicates that soils high in organic matter have greater 'residual' shrinkage than soils low in this respect, and agrees with the findings of Haines (8) who obtained the highest 'residual' shrinkage, 18.2% volume, on drying peat soil below 50.5% moisture, and only 2.2% in Rothamsted loam below 18.7% moisture. The low 'residual' shrinkage of Fallis gray wooded soil indicates a loss of colloidal material from the soil, probably by leaching, since it is a badly leached soil.

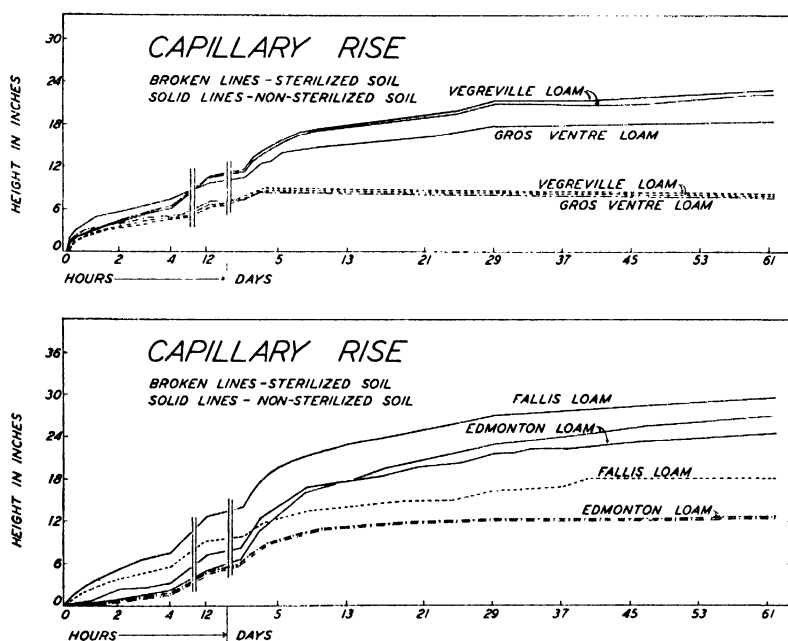
## STICKY POINT

The 'sticky point' or point of maximum stickiness appears to be reached at a lower moisture content in sterilized soils than in the unsterilized. It was reached when Edmonton unsterilized soil was brought to 60% moisture (air-dry basis) and sterilized soil was brought to 58%. At a given moisture content, approximately 40% moisture, Edmonton sterilized was more sticky than unsterilized soil. Vegreville, Gros Ventre, and Fallis unsterilized attained sticky points at 52, 50, and 33% of moisture.

On cultivating the soils in the flasks incubated for nitrification, it was observed that those that were kept sterile for a long time became quite sticky upon repeated addition of water to maintain weight for optimum moisture, while the unsterilized and the sterilized recontaminated, with the same moisture content, were not inclined to become sticky.

## CAPILLARY RISE

Figs. 4 and 5 show the results of the capillary rise experiment. Consistent and definite differences were found. In all cases the capillary rise after the first 12 hr. was greater in the unsterilized soils. Differences became



FIGS. 4 and 5. Capillary rise in non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil, and Fallis gray wooded soil.

apparent even after 15 min. in Fallis and Gros Ventre soils. The experiment was carried on for 58 days and the highest rise in unsterilized soil at the end of the experiment was obtained in Fallis soil, the next highest in Edmonton

soil, and the lowest in Gros Ventre. In the sterilized soils the rise was in the same order, although not as high. After 58 days the sterilization had decreased capillary rise by about 49, 62, 53, and 37%, respectively in the Edmonton, Vegreville, Gros Ventre, and Fallis soils.

The differences in capillary rise of water in the different soils, and between sterilized and unsterilized soils, are probably due mainly to differences in texture, quantity, and character of colloidal material present. The quantity of soluble matter, including soluble organic matter, is definitely increased by steaming. Pickering (14) and Lyon and Bizzell (11) found that water soluble matter was increased by steaming.

### pH VALUES

In this investigation, as shown in Table II, no significant differences were found in pH values, as measured by the quinhydrone electrode, between sterilized and unsterilized soils after the sterilized soils had been autoclaved

TABLE II  
HYDROGEN ION CONCENTRATION OF FOUR STERILIZED AND NON-STERILIZED ALBERTA SOILS, EXPRESSED AS pH  
(Determined by the quinhydrone electrode)

	Sterilized				Non-sterilized			
	1 min.	5 min.	15 min.	30 min.	1 min.	5 min.	15 min.	30 min.
Edmonton	6.0	6.1	6.1	6.0	6.3	6.1	6.2	6.0
black	6.1	6.1	6.1	6.1	6.3	6.1	6.3	6.0
park	6.0	6.1	6.1	6.1	6.2	6.2	6.1	6.2
Average	6.0	6.1	6.1	6.1	6.3	6.1	6.2	6.1
Vegreville	6.3	6.1	6.1	6.2	6.2	6.2	6.1	6.2
black	6.2	6.1	6.1	6.2	6.2	6.1	6.0	6.2
park	6.2	6.1	6.1	6.2	6.3	6.1	6.2	6.1
Average	6.2	6.1	6.1	6.2	6.2	6.1	6.1	6.2
Gros Ventre	5.8	5.7	5.8	5.8	6.0	5.8	5.9	5.9
brown	5.8	5.8	5.8	5.7	5.9	5.8	5.9	5.9
prairie	5.7	5.7	5.8	5.7	5.8	5.8	5.9	5.9
Average	5.8	5.7	5.8	5.7	5.9	5.8	5.9	5.9
Fallis	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.1
gray	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
wooded	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
Average	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2

twice, on successive days, once for three-quarters of an hour and the second time for one-half hour at 120° C. Titratable or total acidity was not determined. Schreiner and Lathrop (17) in 1912 found an increase in titratable acidity when they steam sterilized a soil for three hours at 135° C.

## WATER SOLUBLE PHOSPHORUS

Water soluble phosphorus was definitely increased in steam sterilized soils, as shown in Table III. In comparison with unsterilized soil, the quantities were about 2.8 times as great in the Edmonton soil, 1.7 times in the Vegreville,

TABLE III

WATER SOLUBLE PHOSPHORUS IN NON-STERILIZED AND STERILIZED ALBERTA SOILS

(Average p.p.m. phosphorus on water-free basis)

Soil	Non-sterilized			Sterilized		
	No. of determinations	P, p.p.m.	Av. deviation from mean	No. of determinations	P, p.p.m.	Av. deviation from mean
Edmonton black park	12	1.67	.47	18	4.69	.48
Vegreville black park	5	4.17	.22	5	7.01	.20
Gros Ventre brown prairie	4	3.16	.05	4	6.72	.05
Fallis gray wooded	4	1.38	.01	4	1.83	.04

*Without ignition*

Vegreville black park	4	2.25	.37	4	3.80	.70
Gros Ventre brown prairie	4	1.00	.05	—	—	—

2.1 times in the Gros Ventre, and there was a slight increase in the Fallis soil. Without the ignition of water extracts to destroy organic matter, the quantities of water-soluble phosphorus were found to be approximately half as great in both unsterilized and sterilized soils.

The increase in water soluble phosphorus content induced by sterilization was large in the soils high in organic matter and relatively small in Fallis soil, which is low in organic matter, suggesting that organic phosphorus compounds are partly broken down or made more soluble and later broken down by ignition.

Schreiner and Lathrop (17) found that steam sterilization increased the solubility of soil constituents, increasing all of the organic constituents that they had isolated from unheated soil, except nucleic acid, which was decomposed.

## EASILY SOLUBLE PHOSPHORUS (SOLUBLE AT pH 3)

The quantities of easily soluble phosphorus in the four Alberta soils (1) unsterilized, (2) sterilized, and (3) sterilized and recontaminated with original soil, throughout an incubation period of 20 weeks following sterilization, are shown in Table IV. In general, greater quantities of easily soluble phosphorus were found in the sterilized and sterilized recontaminated black Edmonton and Vegreville soils than in the unsterilized. In the Gros Ventre

TABLE IV

EASILY SOLUBLE PHOSPHORUS (SOLUBLE AT pH 3) IN NON-STERILIZED, STERILIZED, AND RECONTAMINATED STERILIZED ALBERTA SOILS

(Average of duplicates, p.p.m. phosphorus on water-free basis)

Soil	Treatment	Period of incubation after sterilizing soil and setting up experiment									Final av.
		Right after ster.	Days		Weeks						
			3	6	2	4	8	12	16	20	
Phosphorus, p.p.m., average of duplicates											
Edmonton black park	Non-sterilized	33	—	32	32	37	46	52	49	55	43
	Sterilized and recontaminated	—	—	40	47	57	52	74	75	80	61
	Sterilized	40	—	42	51	56	56	77	70	63	59
Vegreville black park	Non-sterilized	46	—	82	86	69	96	91	86	108	88
	Sterilized and recontaminated	—	—	78	89	93	125	123	103	113	103
	Sterilized	60	—	83	99	83	103	109	104	110	99
Gros Ventre brown prairie	Non-sterilized	56	48	48	46	56	54	98	56	57	58
	Sterilized and recontaminated	—	48	49	43	53	61	107	61	67	61
	Sterilized	62	56	54	48	52	55	82	53	61	59
Fallis gray wooded	Non-sterilized	87	76	83	82	92	100	115	76	100	90
	Sterilized and recontaminated	—	85	86	82	103	102	120	91	105	97
	Sterilized	99	90	91	82	94	106	125	84	101	97

brown soil, which is low in organic matter, the differences were insignificant, and in the Fallis gray soil, which is also low in organic matter, the differences were probably insignificant, because, while close agreement was obtained in reading the duplicates of a single determination, a variation of 10 p.p.m. was often obtained in separate determinations. In the final averages the increases of sterilized over unsterilized were about 37% in the Edmonton soil, 12% in the Vegreville, 8% in the Fallis, and if anything a little more in the sterilized recontaminated.

Increases in soluble phosphorus found right after sterilization must be attributed to changes brought about by steaming. Increases with time of incubation may be attributed to microbiological activity in the unsterilized

and the sterilized recontaminated soils, following remoistening and cultivation, but this is doubtful because the sterilized soils that were not recontaminated also showed an increase in easily soluble phosphorus.

Darbishire and Russell (7) found that plants grown on partially sterilized soil utilized about twice as much phosphorus as plants grown on normal soil, and that the percentage of phosphorus in plants grown on partially sterilized is much higher than on normal soil. Baldwin (1) found more easily soluble phosphorus in sterilized than in unsterilized soil in the greenhouse.

### NITRIFICATION

The results of the two nitrification experiments are shown in Figs. 6, 7, 8, and 9. Greatest nitrification occurred in Vegreville and Edmonton soils, less in Gros Ventre, and least of all in Fallis soil; that is to say, the higher the organic matter content of the soil, the greater the nitrification.

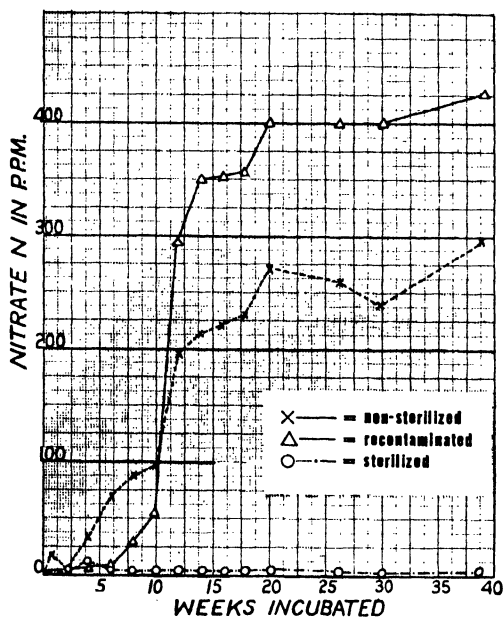


FIG. 6. Nitrification in non-sterilized, recontaminated sterilized, and sterilized Edmonton black park soil.

In all cases the nitrate content remained practically constant and generally very low in the sterilized soils throughout the 39 weeks of incubation. Nitrate accumulated in all of the unsterilized soils. Nitrification was slower at first in the sterilized recontaminated soil but after about 10 weeks, nitrate accumulated more rapidly in the sterilized recontaminated than in the unsterilized. The increase in the rate of nitrification in the sterilized recontaminated is evidently related to the ammonia content of the soil, because the ammonia that had accumulated in these soils began to decrease rapidly in most cases at about 10 weeks or a little earlier.

These results indicate that either the nitrifying organisms (*Nitrosomonas* and *Nitrobacter*) take a long time to become active when re-introduced into

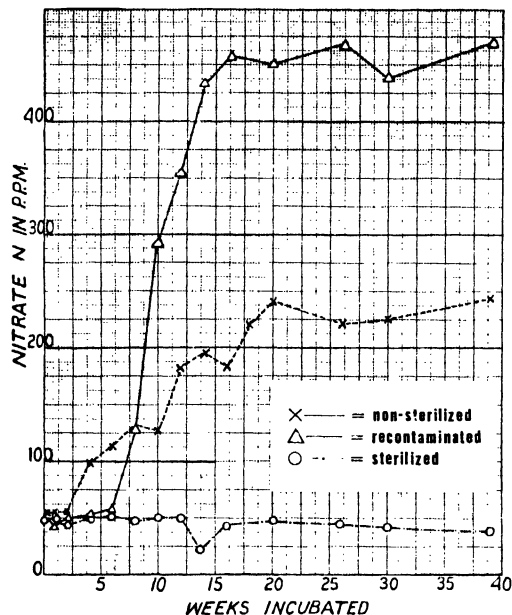


FIG. 7. Nitrification in non-sterilized, recontaminated sterilized, and sterilized Vegreville black park soil.

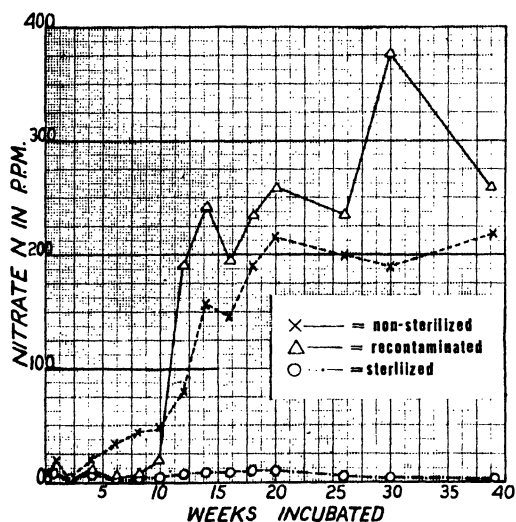


FIG. 8. Nitrification in non-sterilized, recontaminated sterilized, and sterilized Gros Ventre brown prairie soil.

steam sterilized soil, or that sterilization in some way alters the chemical composition of the soil, making it unfavorable for their development until other micro-organisms have had time to overcome the inhibitory factors.

Schreiner and Lathrop (17) found an increase in a large number of organic nitrogen compounds following steam sterilization, and this may account, in part at least, for the greater nitrification in the recontaminated soils. Presumably the same micro-organisms as were present in the unsterilized soil are active, though certain groups may develop more rapidly for a time because of the altered chemical and physical conditions in sterilized soils.

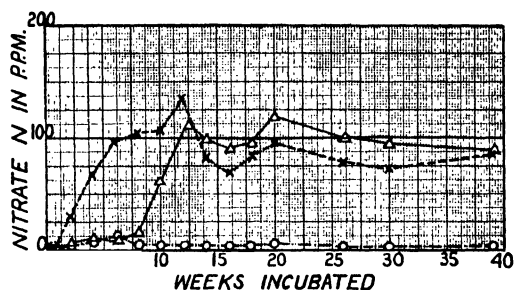


FIG. 9. Nitrification in non-sterilized, recontaminated sterilized, and sterilized Fallis gray wooded soil; X — X = non-sterilized; Δ — Δ = recontaminated; O — O = sterilized.

#### AMMONIFICATION

The greatest ammonification or accumulation of ammonia occurred in the sterilized soil recontaminated with original soil, as shown in Figs. 10 to 13. In the first four to six weeks of incubation, a steady increase in ammonia took place in three out of four of the recontaminated soils, rising to a high peak,

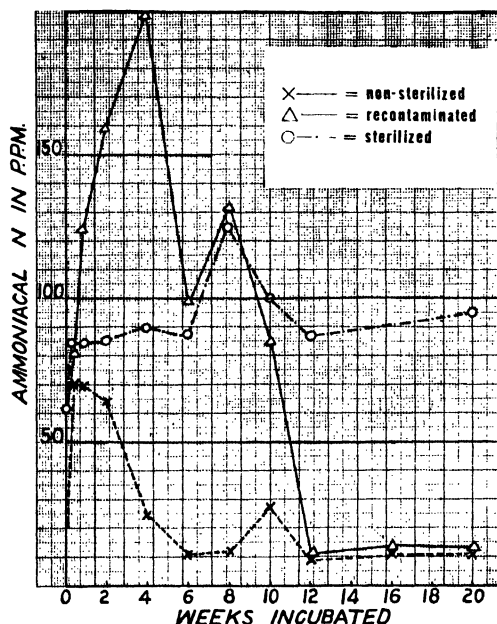


FIG. 10. Ammonification in non-sterilized, recontaminated sterilized, and sterilized Edmonton black park soils.



as may be seen on Figs. 10, 11, and 12. This increase was followed by a rapid return to about the same low level as that of the unsterilized, at about 12

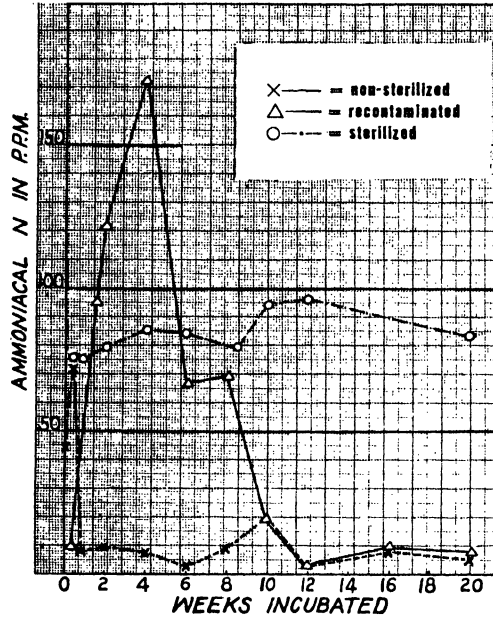


FIG. 11. Ammonification in non-sterilized, recontaminated sterilized, and sterilized Vegreville black park soils.

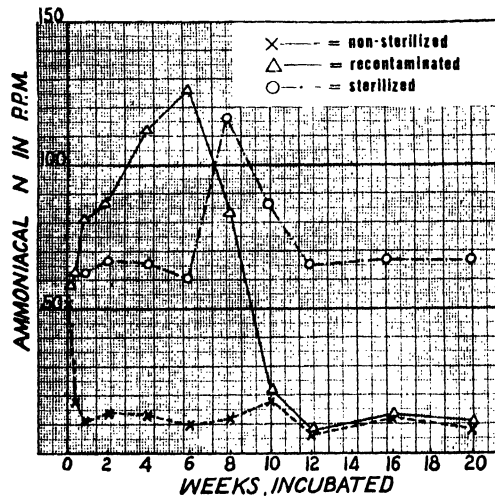


FIG. 12. Ammonification in non-sterilized, recontaminated sterilized, and sterilized Gros Ventre brown prairie soil.

weeks. However, in the case of recontaminated Fallis soil the accumulation of ammonia was relatively small, and it took longer to return to the normal low level. The accumulation of ammonia was roughly proportional to the

organic matter content of the soil: Edmonton, 198 p.p.m. of ammoniacal nitrogen; Vegreville, 172; Gros Ventre, 126; and Fallis, 63 p.p.m.

During the first few days of the experiment there was a temporary increase of ammonia in the unsterilized soils. This was probably the result of bringing

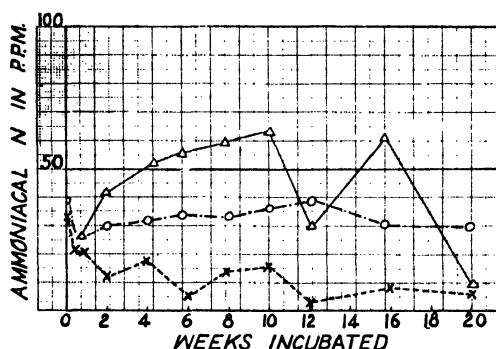


FIG. 13. Ammonification in non-sterilized, recontaminated sterilized, and sterilized Fallis gray wooded soil; X ——— X = non-sterilized; Δ ——— Δ = recontaminated; O ——— O = sterilized.

to optimum conditions of moisture and temperature soils that had been kept air-dry, thus renewing the activity of ammonifying micro-organisms. The nitrifiers apparently do not become active as quickly as the ammonifiers when air-dry soil is remoistened, but after a short period of time they convert nearly all of the available ammonia to nitrate.

The ammonia content of the sterilized soils remained at a fairly constant level above that of the unsterilized. This was probably due to the production of ammonia compounds that were absent before heating (17), but the fluctuations from this more or less constant level for each soil were probably due to contamination from the air, since each flask was opened every week or 10 days in order to add sterilized water. Great care was exercised, when watering flasks, to prevent recontamination, by flaming the mouth of each flask and keeping it open just long enough to add water. Nevertheless the cotton plug through which was placed a stirring rod for cultivating and mixing the soil after watering became loose in some flasks after repeated cultivation.

A shorter experiment on ammonification was also set up as a duplicate of the longer experiment, and ammonia was determined after three and six days, two weeks, and four weeks after sterilization. The results of this experiment are not given because the figures show the same trends as those of the first experiment.

Since the method used to determine ammonia involved the use of magnesium oxide to displace ammonia on distillation of soil extract, it was thought advisable to find out whether magnesium oxide would break down dissolved organic matter and liberate ammonia in stages rather than cause it to be given off completely in one stage. The distillations were carried out under uniform conditions for 45 min. from first boiling, during which time approximately

250 cc. of distillate were collected. When the distillation was prolonged for 45 min., a very small amount of additional ammonia nitrogen was obtained: about 4 p.p.m. as an average of 30 determinations.

For example, in one case more than 90 p.p.m. of ammoniacal nitrogen were distilled off in 10 min., 135 in 15 min., and 180 in 20 min., when the total was less than 200 p.p.m. Distilling for 45 min., from the first boiling, would therefore allow more than enough time for displacement of readily displaceable ammonia.

### SULPHOFICATION

Generally speaking there was some increase in water soluble sulphate in all four unsterilized soils on incubation, as shown in Table V, and large increases in all sterilized recontaminated soils, except Fallis. However, the sterilized

TABLE V

SULPHOFICATION IN NON-STERILIZED, STERILIZED, AND RECONTAMINATED  
STERILIZED ALBERTA SOILS

(Average of duplicates, p.p.m. sulphate on water-free basis)

Soil	Treatment	Period of incubation after sterilizing soil and setting up experiment				
		Right after ster.	Weeks			
			2	4	8	12
Sulphate, p.p.m., average of duplicates						
Edmonton black park	Non-sterilized	71	64	66	69	79
	Sterilized and recontaminated	98	103	94	126	223
	Sterilized	96	114	79	124	141
Vegreville black park	Non-sterilized	76	68	70	126	101
	Sterilized and recontaminated	136	123	100	158	188
	Sterilized	122	147	85	124	152
Gros Ventre brown prairie	Non-sterilized	42	52	—	89	80
	Sterilized and recontaminated	70	68	86	105	106
	Sterilized	110	102	86	108	126
Fallis gray wooded	Non-sterilized	24	42	81	38	39
	Sterilized and recontaminated	50	42	54	57	52
	Sterilized	43	58	36	47	49

soils also showed some fluctuations and apparent increases in water soluble sulphate on incubation, and the results as a whole are not entirely consistent.

Sterilization alone considerably increased the sulphate content of each soil, as shown in the first column of Table V, but this may be due merely to an increase in soluble organic matter, which on ignition gives higher sulphate

TABLE VI  
WATER SOLUBLE SULPHATES WITH AND WITHOUT IGNITION  
(Average of duplicates, p.p.m. SO<sub>4</sub> on water-free basis)

Soil (incubated 12 weeks)	Treatment	Without ignition, SO <sub>4</sub> p.p.m.	With ignition, SO <sub>4</sub> p.p.m.
Edmonton black parkland	Non-sterilized	49	79
	Sterilized and recontaminated	185	222
	Sterilized	81	140
Vegreville black parkland	Non-sterilized	64	100
	Sterilized and recontaminated	127	187
	Sterilized	95	151
Gros Ventre brown prairie	Non-sterilized	45	79
	Sterilized and recontaminated	83	105
	Sterilized	70	125
Fallis gray wooded	Non-sterilized	18	38
	Sterilized and recontaminated	23	51
	Sterilized	15	48

results. Increases in sulphate on incubation are probably brought about by micro-organisms that oxidize proteins and other forms of organic matter that contain sulphur.

A comparison of the amounts of water soluble sulphate found with and without ignition of evaporated extracts is shown in Table VI. Without ignition only about two-thirds as much sulphate was obtained as with ignition, indicating that part of the sulphate obtained on ignition comes from soluble organic matter.

### Acknowledgments

The writers wish to express their appreciation to Dr. F. A. Wyatt, Head of the Department of Soils, for counsel and support of the work, to Dr. V. Ignatieff and other members of the Department of Soils for helpful suggestions, and to the National Research Council for the financial assistance that made this investigation possible.

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## THE FESCUE GRASSLAND OF ALBERTA<sup>1</sup>

E. H. MOSS<sup>2</sup> AND J. A. CAMPBELL<sup>3</sup>

### Abstract

A distinction is drawn between the virgin fescue prairie described as the *Festuca scabrella* association, and the fescue grassland produced by mowing for hay or by grazing. The virgin or climax association, dominated by *Festuca scabrella*, is characterized by some 150 species of higher plants. Various of the associated species, notably a few of the grasses, increase greatly in frequency and coverage when the prairie is used as hay meadow or as pasture, while the fescue shows a corresponding decline. Where grazing has been heavy the fescue has virtually disappeared. The fescue association is considered in relation to *Stipa*, poplar, and other contiguous plant communities. The name 'submontane' for the fescue prairie of Alberta and Saskatchewan is inappropriate. The fescue association is believed to have been associated with the formation of the black soils of Alberta, including the soils of those black and gray-black zones occupied in recent times by wooded vegetation. The bulk of the organic matter in the black soils has apparently derived from a single species of grass, viz. *Festuca scabrella*. An understanding of climax communities and successional relationships is basic to a proper classification of range land for lease purposes, and to sound long-term administrative policy for this land.

### Introduction

Many parts of the vast prairie formation of North America have been carefully investigated by ecologists, but the more northern areas along the foothills of Alberta and on the southern fringe of the boreal forest in Canada have received little critical study. Weaver and Clements (9) include these northern areas in their "Mixed Prairie" association though they do not recognize *Festuca scabrella*, actually the dominant species of these areas, as one of the chief grasses of the association. Clements and Clements (3) make brief reference to a "submontane" type of mixed prairie extending from northern California and Nevada through all of nonforested Idaho and for some distance into Montana and Utah. They point out that the submontane type has its counterpart at moderate levels in central Alberta and Saskatchewan and mention the chief grasses, including *Festuca scabrella*, of this prairie type. Clarke *et al.* (1) following Clements adopt the rather inappropriate name "submontane" for the fescue grassland of the foothill and grove belt regions of western Canada. Moss (5) describes the grassland of the grove belt or parkland in Alberta as the "northern prairie" and regards *Festuca scabrella* as one of the chief dominants. For a limited area in southwestern Alberta, Moss (6) recognizes a *Festuca-Danthonia* association. The present paper presents additional data for the fescue grassland of Alberta, with a view to clarifying the ecological concepts and nomenclature involved. The paper deals also with certain practical aspects of grassland ecology, especially problems of land-use classification and the successional changes

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induced by utilization. Some consideration is given to the matter of vegetational change in response to major climatic shifts and to the role of vegetation in soil formation. For a discussion of the climate, soil zones, and topography of the region, reference may be made to earlier papers (1, 2, 5, 6).

### Methods

During the past three years we have been able to study more extensively and more critically than hitherto, representative relict prairie areas in the foothill and parkland regions of Alberta. Relict areas are now exceedingly rare and soon will have become practically non-existent unless measures are taken promptly to preserve some of those that still remain. One of the best of these areas, located some 15 miles southeast of Cardston was being broken even as we were making our records. For comparison with the relict samples, which we regard as essentially virgin and climax prairie, we studied near-by grassland that had been moderately grazed or mowed for a number of years. Heavily grazed areas, by no means uncommon, were also examined. Special attention was paid to vegetational variations associated with the more obvious differences in soil and topography.

For the various areas studied the species were listed and their prevalence recorded by use of the following symbols and by supplementary notes.

Symbols shown in Tables I, II, III.

d . . . . .	Dominant
a . . . . .	Abundant
f . . . . .	Frequent, quite common
s . . . . .	Scattered, fairly common
o . . . . .	Occasional, uncommon
r . . . . .	Rare
vr . . . . .	Very rare

Each of these symbols records an estimate of the dispersion, density, and coverage of a species.

Special attention was given to the frequency and coverage of grass species. Quantitative records for statistical treatment were not made, though the observations reflect a background of previous experience in numerical recording of vegetation cover. Certain quantitative results for utilized fescue grassland under the name "submontane mixed prairie" have been published (1).

Sample areas in each of the following regions (Fig. 1) were studied: Edmonton, Vermilion, Alix, Drumheller, Hand Hills, Calgary-Cochrane, Porcupine Hills, Pincher-Waterton, Cardston. In Table I, the first five of these regions are referred to as "north", the last four as "south". The entries in Tables I, II, and III represent a synthesis of the records for the various areas and regions. Since the sample areas of the different regions varied considerably in number, size, and suitability, and since some regions were studied more intensively than others, the results are not strictly comparable in every respect;

but they do provide a fairly accurate over-all picture of the range and relative importance of the different species in the fescue grassland.

### The *Festuca scabrella* Association

This association is believed to have comprised the greater part of the native or virgin grassland found in the black soil zones of Alberta (Fig. 1). In addition to grassland these zones had large areas of poplar and other forest.

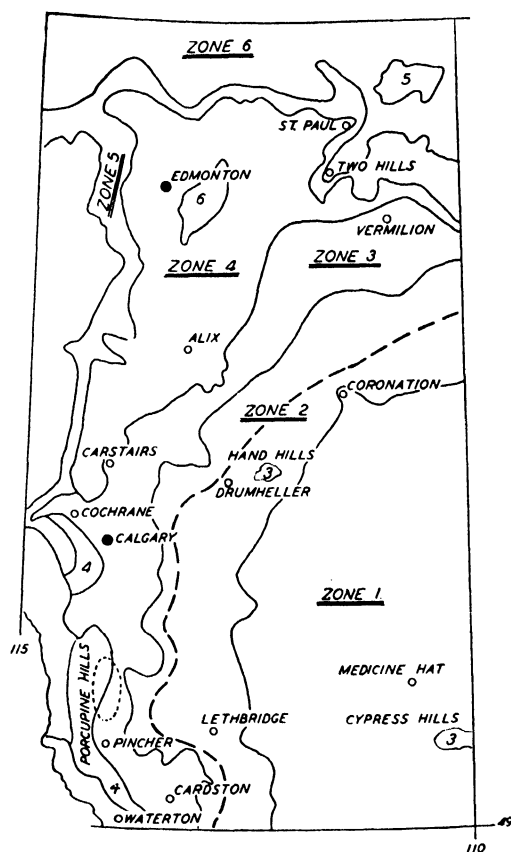


FIG. 1. Map of central and southern Alberta showing soil zones and the approximate tension zone (heavy broken line) between climax *Festuca* and *Stipa* associations. Soil zones per Wm. Odynsky.

Zone 1—light brown soil.  
Zone 2—dark brown soil.  
Zone 3—shallow black soil.

Zone 4—deep black soil.  
Zone 5—gray-black (transition) soil.  
Zone 6—gray (wooded) soil.

Extending approximately through the middle of the black soil region was a broad transition belt of vegetation, the grove belt or parkland, consisting of patches of prairie and aspen poplar vegetation (5). For reasons given below, the *Festuca scabrella* association is thought to have preceded forest on black soil areas recently characterized by trees. It is believed too that this association originally dominated in the transition gray-black soil zone currently



covered by forest. In the dark brown soil zone, the fescue association was intermixed with a *Stipa* association, the climax prairie of the light brown soil zone. The broken line shown on the map (Fig. 1) in the dark brown soil zone is taken to represent approximately the middle of the natural tension zone between the *Festuca* and *Stipa* associations.

In nearly all parts of the association, *Festuca scabrella* grows almost to the exclusion of other higher plants. This grass forms large bunches or tussocks from 1 to 3 ft. high and 10 to 20 in. in diameter. Between the tussocks the 'characteristic' associated species make relatively feeble growth. Only locally where edaphic conditions are somewhat exceptional does the fescue lose its dominance, e.g. on a gravel bank, or where a burrowing animal has disturbed the soil. •The role of grazing animals in the natural biome is discussed below. While *Festuca scabrella* is undoubtedly the dominant species of the association, *Danthonia Parryi* appears to be a local dominant of considerable importance in restricted areas, especially on shallow soils of rocky and gravelly slopes. Since *D. Parryi* seems also to replace *F. scabrella* under grazing, the exact role of this grass in the virgin grassland is difficult to determine, and further investigation is needed. For the present, it seems best to regard *D. Parryi* as forming an edaphic climax within the *F. scabrella* association. The earlier proposal (6) of a *Festuca-Danthonia* association, while descriptive of typical utilized prairie of southwestern Alberta, does not denote the virgin or climax grassland of that region.

As set forth in Table I the *Festuca scabrella* association includes approximately 150 species of higher plants. There are 20 grasses, 3 sedges, 10 shrubs

TABLE I  
SPECIES OF THE *Festuca scabrella* ASSOCIATION

	Through- out the range	North only	South only	Change with grazing
<i>Grasses</i>				
<i>Agropyron dasystachyum</i> (Hook.) Scribn.	r			o-s
" <i>Griffithsii</i> Scribn. & Smith			r	o-s
" <i>spicatum</i> (Pursh) Scribn. & Smith			r	o
" <i>trachycaulum</i> (Link) Malte				
var. <i>unilaterale</i> (Cassidy) Malte	s			f-a
" <i>glaucum</i> (Pease & Moore) Malte				
" <i>typicum</i> Fern.	r			o
<i>Agrostis scabra</i> Willd.	r			o
<i>Avena Hookeri</i> Scribn.	r			o-s
<i>Bromus anomalus</i> Rupr.		vr		
<i>Calamagrostis montanensis</i> Scribn.	r			o-s
<i>Danthonia intermedia</i> Vasey	r			o-f
" <i>Parryi</i> Scribn.			o-d	o-d
<i>Festuca idahoensis</i> Elmer			f	s-a
" <i>scabrella</i> Torr.	d			a-f
<i>Koeleria cristata</i> (L.) Pers.	o			f
<i>Poa Canbyi</i> (Scribn.) Piper (incl. <i>Poa lucida</i> Vasey)			r	o-s
" <i>Cusickii</i> Vasey			r	o-s

TABLE I—Continued

SPECIES OF THE *Festuca scabrella* ASSOCIATION—Continued

	Through- out the range	North only	South only	Change with grazing
<i>Grasses—Concluded</i>				
<i>Poa interior</i> Rydb.	r			o-s
<i>Stipa columbiana</i> Macoun			r	o-s
“ <i>Richardsonii</i> Link			r	o
“ <i>spartea</i> Trin.	r			r
“ <i>spartea</i> var. <i>curtiseta</i> Hitchc.	o			s-f
“ <i>viridula</i> Trin.	r			o-s
<i>Sedges</i>				
<i>Carex Eleocharis</i> Bailey			o	f
“ <i>heliophila</i> Mack.	o			f
“ <i>obtusata</i> Lilj.	r			s
<i>Shrubs</i>				
<i>Amelanchier alnifolia</i> Nutt.	r			s
<i>Dasiphora fruticosa</i> (L.) Rydb.			o	f
<i>Elaeagnus argentea</i> Pursh	r			
<i>Prunus melanocarpa</i> (A. Nels.) Rydb.	r			o
<i>Ribes selosum</i> Lindl.	r			
<i>Rosa arkansana</i> Porter (incl. <i>R. alcea</i> Greene & <i>R. suffulta</i> Greene)	o			s
“ <i>Woodsii</i> Lindl. (incl. <i>R. Macounii</i> (Greene)	r			s
<i>Salix Bebbiana</i> Sarg.	s			
“ “ var. <i>perrostrata</i> Schneid.		vr		
<i>Spiraea alba</i> Du Roi				
<i>Symphoricarpos occidentalis</i> Hook.	s			
<i>Forbs, etc.</i>				
<i>Achillea Millefolium</i> L.	o			s
<i>Agoseris glauca</i> (Nutt.) Greene (incl. var. <i>dasycephala</i> (T. & G.) Jepson)	r			
<i>Allium cernuum</i> Roth	r			
“ <i>textile</i> Nels. & Macbr.	r			
<i>Androsace puberulenta</i> Rydb.	r			o
<i>Anemone cylindrica</i> A. Gray	r			o
“ <i>globosa</i> Nutt.	o			s
<i>Antennaria aprica</i> Greene	r			o
“ <i>campestris</i> Rydb.	r			o
“ <i>concinna</i> A. Nels.		vr		
“ <i>nitida</i> Greene	o			f
“ <i>pulcherrima</i> (Hook.) Greene			vr	o
“ <i>rosea</i> (D. C. Eat.) Greene	r			s
<i>Arabis divaricarpa</i> A. Nels.	r			
“ <i>hirsuta</i> (L.) Scop.	o			
“ <i>Holboellii</i> Hornem.				
var. <i>retrofracta</i> (Grah.) Rollins			r	
<i>Arnica Chamissonis</i> Less.	r			
<i>Artemisia camporum</i> Rydb.	r			o

TABLE I—Continued

SPECIES OF THE *Festuca scabrella* ASSOCIATION—Continued

	Through- out the range	North only	South only	Change with grazing
<i>Forbs, etc.</i> —Continued				
<i>Artemisia frigida</i> Willd.	o			f
“ <i>gnaphalodes</i> Nutt.	o			f
<i>Aster adsurgens</i> Greene ( <i>A. crassulus</i> Rydb.)	r			o
“ <i>alpinus</i> L. ( <i>A. culminis</i> A. Nels.)			r	
“ <i>commutatus</i> (T. & G.) A. Gray	o			s
“ <i>laevis</i> L. (incl. <i>A. Geyeri</i> )	o			s
<i>Astragalus bisulcatus</i> (Hook.) A. Gray			r	o
“ <i>Drummondii</i> Dougl.	r			
“ <i>flexuosus</i> Dougl.	r			
“ <i>goniatus</i> Nutt.	o			
“ <i>pectinatus</i> (Hook.) Dougl.			r	o
“ <i>striatus</i> Nutt.	o			
<i>Balsamorhiza sagittata</i> (Pursh) Nutt.			o	s
<i>Campanula rotundifolia</i> L.	r			
<i>Castilleja</i> ? <i>lauta</i> A. Nels.	}			
“ <i>lutescens</i> (Greenm.) Rydb.			r	
“ <i>lutea</i> Heller				
<i>Cerastium arvense</i> L.	o			f
<i>Cheirinea inconspicua</i> (S. Wats.) Rydb.	r			
<i>Cirsium Flodmanii</i> (Rydb.) Arth.	r			o
“ <i>undulatum</i> (Nutt.) Spreng.		vr		
<i>Comandra pallida</i> A. DC.	r			s
<i>Crepis intermedia</i> A. Gray			vr	
<i>Dodecatheon Cusickii</i> Greene			r	
<i>Draba nemorosa</i> L.	r			
<i>Drymocallis agrimonioides</i> (Pursh) Rydb.	r			o
<i>Erigeron caespitosus</i> Pursh	o			f
“ <i>formosissimus</i> Greene			vr	
“ <i>glabellus</i> Nutt.	r			o
“ “ var. <i>pubescens</i> Hook.	o			s
<i>Fragaria glauca</i> (S. Wats.) Rydb.	r			s
<i>Fritillaria pudica</i> (Pursh) Spreng.			vr	
<i>Gaillardia aristata</i> Pursh	r			s
<i>Galium boreale</i> L.	o			f
<i>Gentiana Amarella</i> L.	r			o
<i>Geranium viscosissimum</i> Fisch. & Mey.			o	s
<i>Habenaria viridis</i> (L.) R. Br.	r			
“ var. <i>bracteata</i> (Muhl.) A. Gray				
<i>Hedysarum alpinum</i> L.	}			
“ var. <i>americanum</i> Michx. ex Pursh		r		o
“ var. <i>philoscia</i> (A. Nels.) Rollins				
“ “ <i>Mackenzii</i> Richards.			o	s
“ <i>sulphurescens</i> Rydb.			r	
<i>Helianthus rigidus</i> (Cass.) Desf.	vr			o
( <i>H. subrhomboides</i> Rydb.)				
<i>Heuchera cylindrica</i> Dougl.	}			
“ var. <i>septentrionalis</i> R. B. L.		r		o
“ ( <i>H. Richardsonii</i> R. Br.)				
“ <i>flabellifolia</i> Rydb.			r	
“ var. <i>typica</i> R. B. L.				
<i>Hieracium canadense</i> Michx.	r			
<i>Lepachys columnifera</i> (Nutt.) Rydb.			r	o
<i>Liatris punctata</i> Hook.			o	s
<i>Lilium philadelphicum</i> L. var. <i>andinum</i> (Nutt.) Ker	r			
<i>Linum Lewisii</i> Pursh			r	o
<i>Lithospermum ruderales</i> Lehm.			o	f

TABLE I—Concluded

SPECIES OF THE *Festuca scabrella* ASSOCIATION—Concluded

	Through- out the range	North only	South only	Change with grazing
<i>Forbs, etc.</i> —Concluded				
<i>Lomatium macrocarpum</i> (H. & A.) C. & R.			r	o
“ <i>simplex</i> (Nutt.) Macbr.			r	o
“ var. <i>leptophyllum</i> (Hook.) Mathias				
“ <i>trinernatum</i> (Pursh) C. & R.			r	
<i>Lupinus argenteus</i> Pursh			vr	o
“ <i>leucopsis</i> Agardh.			o	f
<i>Lychnis Drummondii</i> S. Wats.	r			
<i>Lygodesmia juncea</i> (Pursh) D. Don.	r			
<i>Orophaca caespitosa</i> (Nutt.) Britt.			vr	
<i>Orthocarpus luteus</i> Nutt.	vr			o
<i>Oxytropis gracilis</i> (A. Nels.) K. Schum.	o			s
“ <i>Macounii</i> (Greene) Rydb.			r	o
“ <i>splendens</i> Dougl.	r			o
“ <i>viscida</i> Nutt.			vr	o
<i>Peltigera</i> spp.	r			o
<i>Penstemon albidus</i> Nutt.			vr	
“ <i>confertus</i> Dougl.			vr	
“ <i>gracilis</i> Nutt.	vr			
“ <i>nitidus</i> Dougl.			vr	o
<i>Petalostemon purpureus</i> (Vent.) Rydb.	r			o
<i>Plantago septata</i> Morris			vr	
<i>Polygonum bistortoides</i> Pursh			r	
“ <i>Douglasii</i> Greene			vr	
<i>Polytrichum juniperinum</i> Willd.	r			
“ <i>piliferum</i> Schreb.	r			
<i>Potentilla gracilis</i> Dougl.	o			
“ <i>Hippiana</i> Lehm.			r	o
“ <i>strigosa</i> Pall.	o			
<i>Ranunculus cardiophyllus</i> Hook.	r			o
“ <i>ovalis</i> Raf.	r			o
<i>Rhinanthus Kryollae</i> Chab.			vr	
<i>Senecio canus</i> Hook.				
“ <i>Purshianus</i> Nutt.	r			o
<i>Senecio columbianus</i> Greene			r	
“ <i>integerrimus</i> Nutt.				
<i>Sieversia ciliata</i> (Pursh) G. Don.	o			f
<i>Silene multicaulis</i> Nutt.			vr	
<i>Sisyrinchium angustifolium</i> Miller	r			o
<i>Smilacina stellata</i> (L.) Desf.	r			o
<i>Solidago decumbens</i> Greene				
var. <i>oreophila</i> (Rydb.) Fern.	o			s
<i>Solidago nemoralis</i> Ait. var. <i>decemflora</i> (DC.) Fern.		r		o
“ <i>missouriensis</i> Nutt. incl. <i>S. glaberrima</i>	o			f
“ <i>Martens</i>				
“ <i>mollis</i> Bartl.			r	o
<i>Spiranthes Romanzoffiana</i> Cham.	vr			
<i>Stellaria longipes</i> Goldie	r			
“ <i>stricta</i> Richards.				
<i>Thalictrum venulosum</i> Trel.	r			o
<i>Thermopsis rhombifolia</i> (Nutt.) Richards.	r			s
<i>Vicia sparsifolia</i> Nutt.			o	s
<i>Viola adunca</i> J. E. Smith	r			o
“ <i>vallicola</i> A. Nels.	vr			
<i>Zizia cordata</i> (Walt.) Koch	r			o
<i>Zygadenus gramineus</i> Rydb.			o	s

and about 115 forbs. A few mosses and lichens are also recorded. Some 25 of the total number are very rare or quite localized, leaving about 125 that may be designated 'characteristic' species. Of these 125 species, some 70 may be described as 'constant', for they are likely to be found in any sizable area (several acres) of the association. In a small sample of the association, such as a few square rods, the number of these 'constants' would probably be not more than 25. These various figures could be considerably increased through more extensive investigation of the association, as also by admitting finer taxonomic divisions in certain of the species groups.

In addition to the species shown in Table I many others (Table II) have been found in fescue grassland but, for various reasons, these are excluded from the *Festuca scabrella* association. These species occur in fescue grassland that has been more or less modified through grazing or mowing, or occupy small special habitats within the prairie. They are characteristically species of other plant communities. It must be emphasized, however, that some of the decisions about the association status of species are quite arbitrary; certain species might be placed either in Table I or Table II. In passing it may be noted that the total number of species for the fescue grassland as listed in Tables I and II is approximately 260.

TABLE II  
SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO  
THE *Festuca scabrella* ASSOCIATION

	Moist or shaded areas	Dry or denuded areas	Change with grazing
<i>Grasses</i>			
<i>Agropyron albicans</i> Scribn. & Smith		r	o
" <i>Smithii</i> Rydb.		o	f
var. <i>molle</i> (Scribn. & Smith) Jones		s	f-a
<i>Andropogon scoparius</i> Michx.		o	f
<i>Bouteloua gracilis</i> (H.B.K.) Lag.		o	f-a
<i>Bromus carinatus</i> Hook. & Arn.			
var. <i>marginatus</i> (Nees) Hitchc.	o		
" <i>ciliatus</i> L.	r		
" <i>Pumpellianus</i> Scribn.	o		
<i>Calamagrostis inexpansa</i> A. Gray	r		
" <i>purpurascens</i> R. Br.	r		
<i>Calamovilfa longifolia</i> (Hook.) Scribn.		r	o
<i>Danthonia californica</i> Boland			
var. <i>americana</i> (Scribn.) Hitchc.	vr		
<i>Deschampsia caespitosa</i> (L.) Beauv.	s		
<i>Elymus Macounii</i> Vasey	r		
<i>Festuca ovina</i> L.		r	
<i>Hierochloa odorata</i> (L.) Wahl.	o		
<i>Muhlenbergia cuspidata</i> (Torr.) Rydb.		o	f
" <i>racemosa</i> (Michx.) B.S.P.	r	r	
" <i>Richardsonis</i> (Trin.) Rydb.	r	r	s
<i>Oryzopsis canadensis</i> (Poir.) Torr.	r		
<i>Poa juncifolia</i> Scribn.		r	o
" <i>palustris</i> L.	s		
" <i>pratensis</i> L.		o	f

TABLE II—Continued

SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO  
THE *Festuca scabrella* ASSOCIATION—Continued

	Moist or shaded areas	Dry or denuded areas	Change with grazing
<b>Grasses—Continued</b>			
<i>Poa secunda</i> Presl.		s	f-a
<i>Schizachne purpurascens</i> (Torr.) Swallen	o		
<i>Stipa comata</i> Trin. & Rupr.		o	f-a
<b>Sedges</b>			
<i>Carex filifolia</i> Nutt.		r	s
“ <i>praegracilis</i> W. Boott	r		
“ <i>pratensis</i> Dewey	r		
“ <i>praticola</i> Rydb.	r		
“ <i>scirpiformis</i> Mack.	r		
“ <i>siccata</i> Dewey		r	s
“ <i>Sprengelii</i> Dewey	vr		
“ <i>xerantica</i> Bailey	r		
<b>Shrubs</b>			
<i>Arctostaphylos uva-ursi</i> (L.) Spreng.		r	o
<i>Artemisia cana</i> Pursh		r	o
<i>Betula glandulosa</i> Michx.	r		
“ <i>microphylla</i> Bunge	r		
<i>Juniperus horizontalis</i> (Moench) Rydb.		r	o
<i>Populus tremuloides</i> Michx.	r		
<i>Salix discolor</i> Muhl.	r		
“ <i>petiolaris</i> Smith	r		
“ <i>pyrifolia</i> Anders.	r		
<b>Forbs, etc.</b>			
<i>Actinea acaulis</i> (Pursh) Spreng. ( <i>Tetranneuris acaulis</i> )		vr	o
<i>Agastache anethiodora</i> (Nutt.) Britt.	r		
<i>Allium Schoenoprasum</i> L. var. <i>sibiricum</i> (L.) Hartm.	r		
<i>Anemone canadensis</i> L.	r		
<i>Apocynum androsaemifolium</i> L.		r	
<i>Arabis Nuttallii</i> Robinson	r		
<i>Artemisia caudata</i> Michx.		r	
<i>Aster coerulescens</i> DC.	r		
<i>Astragalus missouriensis</i> Nutt.		r	
<i>Besseyia wyomingensis</i> (A. Nels.) Rydb.		r	
<i>Bupleurum americanum</i> Coult. & Rose		r	
<i>Camassia esculenta</i> Lindl.	r		
<i>Ceratodon purpureus</i> (L.) Brid.		o	s
<i>Chrysopsis</i> spp.		o	s
<i>Cladonia</i> spp.		o	f
<i>Collomia linearis</i> Nutt.	r		
<i>Crepis runcinata</i> (James) T. & G.	r		
<i>Cryptantha Bradburiana</i> Payson		vr	
<i>Dodecatheon pauciflorum</i> (Durand) Greene	r		
<i>Epilobium angustifolium</i> L.	r		

TABLE II—Concluded

SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO  
THE *Festuca scabrella* ASSOCIATION—Concluded

	Moist or shaded areas	Dry or denuded areas	Change with grazing
<i>Forbs, etc.</i> —Continued			
<i>Erigeron speciosus</i> (Lindl.) DC.	vr		
“ <i>subtrinervis</i> Rydb. ( <i>E. conspicuus</i> Rydb.)	vr		
<i>Eriogonum flavum</i> Nutt.		r	o
<i>Gaura coccinea</i> Pursh		r	s
<i>Geum macrophyllum</i> Willd.	r		o
“ var. <i>perincisum</i> (Rydb.) Raup			
“ <i>strictum</i> Soland	r		o
<i>Glycyrrhiza lepidota</i> (Nutt.) Pursh	r		o
<i>Grindelia perennis</i> A. Nels.		r	o
<i>Gutierrezia diversifolia</i> Greene		o	f
<i>Hedysarum boreale</i> Nutt.		r	o
“ var. <i>typicum</i> Rollins			
“ <i>boreale</i> Nutt.		r	o
“ var. <i>cinerascens</i> (Rydb.) Rollins			
<i>Helianthus subtuberosus</i> Bourg.	vr		
<i>Hieracium griseum</i> Rydb.	vr		
<i>Hymenopappus filifolius</i> Hook.		r	
<i>Hymenoxys Richardsonii</i> (Hook.) Cockerell		o	f
<i>Juncus balticus</i> Willd. var. <i>montanus</i> Engelm.	r		
“ <i>Vaseyi</i> Engelm.	vr		
<i>Lathyrus ochroleucus</i> Hook.	r		
<i>Linum rigidum</i> Pursh		r	
<i>Luzula campestris</i> DC.			
“ var. <i>multiflorus</i> (Ehrh.) Celak	vr		
<i>Malvastrum coccineum</i> (Pursh) A. Gray		r	s
<i>Mentha canadensis</i> (L.) var. <i>glabrata</i> Benth.	vr		
<i>Monarda menthaefolia</i> Benth.	r		
<i>Musineon divaricatum</i> (Hook.) Raf.			
“ var. <i>Hookeri</i> (Nutt.) Mathias		r	o
<i>Nabalus racemosus</i> (Michx.) DC.	vr		
<i>Orobanche fasciculata</i> Nutt.		vr	
<i>Oxytropis deflexa</i> (Pall.) DC.	r		
<i>Parnassia multiseta</i> (Ledeb.) Fern. ( <i>P. palustris</i> L.)	r		
<i>Paronychia sessiliflora</i> Nutt.		o	s
<i>Penstemon procerus</i> Dougl.	r		
<i>Perideridia Gairdneri</i> (H. & A.) Mathias			
( <i>Carum Gairdneri</i> )	r		
<i>Petalostemon candidus</i> (Willd.) Michx.		r	o
<i>Phlox Hoodii</i> Richards.		o	f
<i>Polygala Senega</i> L.	vr		
<i>Potentilla camporum</i> Rydb.	r		
“ <i>concinna</i> Richards.		r	o
“ <i>viridescens</i> Rydb.	vr		
<i>Selaginella densa</i> Rydb.		o	f
<i>Sideranthus spinulosus</i> (Pursh) Sweet		r	o
<i>Solidago gigantea</i> Ait. var. <i>leiophylla</i> Fern.	r		
“ <i>gilvocanescens</i> (Rydb.) Smyth			
( <i>S. canadensis</i> var. <i>gilvocanescens</i> )	r		
“ <i>rigida</i> L. ( <i>Oligoneuron canescens</i> )	r		o
<i>Stachys scopulorum</i> Greene	r		
<i>Steironema ciliatum</i> (L.) Raf.	r		
<i>Vicia americana</i> Muhl.	vr		
<i>Zygadenus elegans</i> Pursh	r		

Of considerable interest is the fact that the *Festuca scabrella* association as it occurs in southern Alberta is much richer in associated species than it is northward and eastward. As shown in Table I approximately 150 species have been recorded for the southern region and slightly over 100 species for the northern portion of the association. Some 50 species found in the south are not recorded for the north, while only four species in the north are not noted for the south. The richer flora of the southern part of the association may be explained primarily in terms of proximity to a variety of other vegetational types, especially of the mountains. Through mountain passes and also southward there is actual continuity with extensions of the Palouse prairie of the northwestern United States. Many migrant species from the west and south have apparently found niches in the fescue association of southern Alberta. Characterized by a wide diversity of edaphic conditions associated with a rough topography, this region would seem to favor the establishment of migrants. The absence of these species northward may be explained in part by the somewhat narrow habitat range for the fescue association there, but mainly in terms of the time element, insufficient time having elapsed since glaciation for these southern species to have reached the north.

As pointed out in an earlier paper (6) notable extensions of Palouse prairie species into our region are *Festuca idahoensis*, *Stipa columbiana*, *Agropyron spicatum*, *Geranium viscosissimum*, *Lithospermum ruderae*, and *Balsamorhiza sagittata*. Of these species, some have spread farther north than others. *Festuca idahoensis*, an important species in southwestern Alberta, and known also to occur eastward in Alberta to the Cypress Hills, extends northward as far as Carstairs, about 35 miles beyond Calgary (Table III). Northward through the Porcupine Hills this fescue becomes gradually less frequent, while beyond the Hills to Calgary and Carstairs it passes from the role of a scattered species to one of rare occurrence in the *Festuca scabrella* association. *Agropyron spicatum* while found chiefly on dry slopes and shallow soils of ridges in the southwestern region occurs sporadically eastward to the Cypress Hills, including stations near Del Bonito, southeast of Cardston. *Balsamorhiza sagittata* seems not to extend north of the Porcupine Hills. *Geranium viscosissimum* and *Lithospermum ruderae* are found at least as far north as Carstairs. Other species of considerable importance in the southern part of the association, but not recorded for the north include: *Lupinus leucopsis*, *Liatris punctata*, *Vicia sparsifolia*, *Zygadenus gramineus*, and *Dasiphora fruticosa*. The last species, the shrubby cinquefoil, is another of the southern species with an extension eastward in the Cypress Hills and also northward a considerable distance—approximately 70 miles north of Calgary.

*Danthonia Parryi* is worthy of a special note. This grass with a range from Alberta to New Mexico seems to have received little attention from ecologists. While apparently unimportant as a range plant in the United States it is a grass of some consequence from Waterton northward through the Porcupine Hills. In the northern part of the Porcupine Hills and beyond Calgary, this



TABLE III

GRASSES AND SEDGES IN APPROXIMATE ORDER OF THEIR IMPORTANCE IN THE FESCUE ASSOCIATION, SHOWING THEIR GEOGRAPHICAL OCCURRENCE AND THE CHANGES IN FREQUENCY AND COVER PRODUCED BY MODERATE GRAZING AND MOWING (SEE FIG. 2)

	A	B	C	D	E	F	G	H	I	*
<i>Festuca scabrella</i>	d	d	d	d	d	d	f-d	f-d	f-d	a-s
" <i>idahoensis</i>						o	o-f	f	f	s-a
<i>Agropyron trachycaulum</i>	s	s	s	s	s	s	s	s	s	f-a
<i>Koeleria cristata</i>	o	o	o	o	o	o	o	o	o	f
<i>Stipa spartea</i> var. <i>curtiseta</i>	r	o	o	o	o	o	o	o	o	s-f
<i>Danthonia intermedia</i>	r	r	r	r	r	r	r	r	r	o-f
" <i>Parryi</i>						o	s	f	f	o-d
<i>Avena Hookeri</i>	r	r	r	r	r	r	r	r	r	o-s
<i>Poa interior</i>	r	r	r	r	r	r	r	r	r	o-s
" <i>Canbyi</i>			r	r	r		r	r	r	o-s
" <i>Cusickii</i>						r	r	r	r	o-s
<i>Agropyron dasystachyum</i>	r	r	r	r	r	r	r	r	r	o-s
" <i>Griffithsii</i>					r	r	r	r	r	o-s
" <i>spicatum</i>							r	r	r	o
<i>Stipa viridula</i>	r	r	r	r	r	r	r			o-s
" <i>columbiana</i>						r	r	r		o-s
" <i>Richardsonii</i>						r	r	r		o
" <i>spartea</i>			r			r	r			r
<i>Calamagrostis montanensis</i>		r	r	r	r	r	r	r	r	o
<i>Agrostis scabra</i>	r	r	r		r	r	r	r	r	o
<i>Bromus anomalus</i>	r		r	r	r	r				r
<i>Carex heliophila</i>	o	r	r	o	r	r	o	o	o	f
" <i>Eleocharis</i>		r			r	r	o	o	o	f
" <i>obtusata</i>	r	r	r	r	r	r	r	r	r	s

A—Edmonton

B—Vermilion

C—Alix

D—Drumheller

E—Hand Hills

F—Calgary—Cochrane

G—Porcupine Hills

H—Pincher—Waterton

I—Cardston

\*—Changes produced by moderate grazing and mowing.

species decreases in frequency. North of Calgary it occurs sporadically as far as the Carstairs region where it becomes rare (Table III).

Previous accounts (1, 5, 6) of the vegetation of our region, while recognizing the importance of *Festuca scabrella*, gave a large place also to certain other grasses, notably, *Agropyron* spp., *Stipa* spp., *Koeleria cristata*, *Poa* spp., *Danthonia* spp., *Festuca idahoensis*, and *Avena Hookeri*, as well as to certain sedges and forbs. These descriptions actually refer to grassland that has been considerably modified through grazing or mowing, and not to the virgin or climax state. In these earlier publications, as well as in many papers on grassland elsewhere, there is no clear indication as to whether the recognized communities are climax or disclimax. As shown in Table I and Fig. 2, the secondary grasses and sedges have a relatively small place in the climax community but become leading species in the modified grassland.

The words 'virgin' and 'climax' used synonymously above in reference to the *Festuca scabrella* association, demand some clarification. 'Virgin' is used in the usual sense of the prairie as white man first found it. 'Climax' has

reference to the grassland community that had developed, or eventually would develop, under climatic conditions prevailing in the region. Both terms take into account the whole biota, including aboriginal man and native grazing animals, and their influence on the grassland. The role of burning as employed by Indian tribes and the effect of bison grazing will be discussed below, but here the view may be expressed that these factors did not markedly disturb the 'virgin' or 'climax' fescue association that had developed in much of the region.

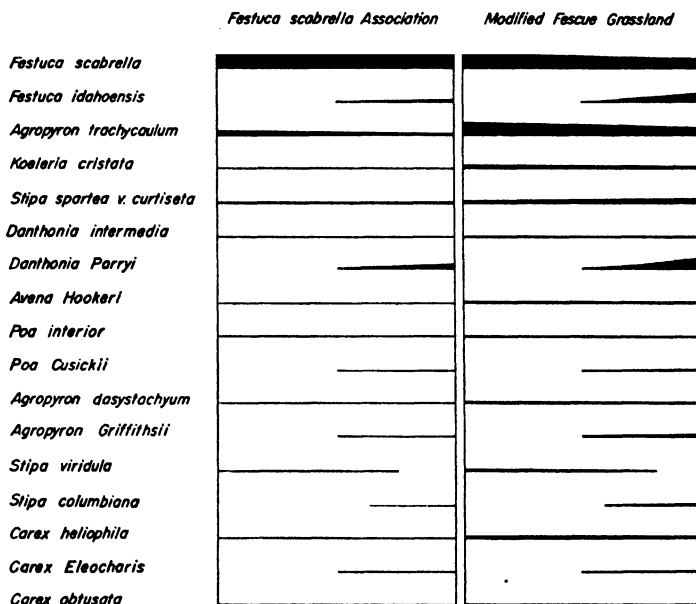


FIG. 2. Graphical representation of the leading grasses and sedges in the *Festuca* association of Alberta, showing the relative abundance of the species from north to south, and the changes produced in this grassland by mowing and by moderate grazing (see Table III).

### Modified Fescue Association

The *Festuca scabrella* association exists in concrete form today only as rare relict areas. Practically all of this association, the virgin fescue grassland, has been either broken or greatly altered through mowing or grazing. Though the unbroken land is often referred to as native or natural prairie, most of it has a plant cover very different from the original. Some of the more important of these changes in the vegetation have been indicated above. They will now be discussed more fully, making reference to the tables and Fig. 2.

Through mowing of the grassland, *Festuca scabrella* has become much smaller in stature and in diameter of tussock but has remained fairly uniformly distributed. Between the tussocks, various of the 'characteristic' species have become prominent, and certain species from other communities of the region have established themselves locally. The more marked changes are seen on the poorer sites, such as stony soil and dry slopes. In these situations,

*Festuca scabrella* may even disappear while other species become abundant, notably *Festuca idahoensis*, *Stipa spartea* var. *curtiseta*, *Agropyron dasystachyum*, *Koeleria cristata*, *Carex* spp., and certain forbs. *Danthonia Parryi* is dominant in localized areas especially on exposed, wind-blown slopes north of Waterton Lakes Park and in the southern part of the Porcupine Hills. This grass should receive further study with a view to determining its role as an edaphic subclimax species, and its occurrence as a grazing or mowing disclimax.

Standard mowing practice for fescue prairie in Alberta is to take a crop of hay in alternate years. Occasionally, two years intervene. When mowed in alternate years a good area will yield about one ton per acre in the year taken. The meadow shown in the photograph (Fig. 3) located northwest of Calgary, had been mowed over a period of 23 years, and had previously been grazed. In 1944 when the photograph was taken, this meadow had not been cut for two years and was yielding a crop of one and a half to two tons per acre. While *Festuca scabrella* was still the chief grass over most of this meadow, various of the other grasses and also many forbs were prominent, with *Danthonia Parryi* locally dominant on some of the slopes and *Stipa spartea* var. *curtiseta* abundant on most of the drier exposed areas.

The effect of grazing of *Festuca scabrella* is somewhat different from that of mowing, in that this grass becomes quite patchy, tending to persist on the more moist situations and where given some protection from grazing animals by shrubby cinquefoil, roses, and other small woody plants. On the better sites, *F. scabrella* is often succeeded to a marked degree by *Agropyron trachycaulum* var. *unilaterale*, while *Koeleria cristata*, *Avena Hookeri*, *Stipa spartea* var. *curtiseta*, and *Danthonia intermedia* may become fairly common. On drier sites, *Agropyron trachycaulum* is likely to become less abundant than *Koeleria*, *Stipa* spp., *Festuca idahoensis*, *Danthonia Parryi*, *Agropyron dasystachyum*, *A. Griffithsii*, and *Poa* spp. The sedges increase markedly with grazing, *Carex heliophila* being found throughout, and *C. Eleocharis* often common in the south (Fig. 2).

Many of the shrubs, forbs, and other species also increase greatly under utilization (Tables I and II). While, in general, the same species tend to become prominent, whether an area is used for hay or for pasture, the shrubs and certain of the forbs, e.g. *Lupinus leucopsis* and *Artemisia* spp., are kept down by mowing and conversely become abundant under grazing.

Heavy or indiscriminate grazing brings about still more marked changes along the lines indicated for moderate grazing. *Festuca scabrella* is soon greatly reduced, or even eliminated, while various of the other grasses, sedges, and other species of the association increase. In some areas, *Carex Eleocharis* has become the chief plant. In other areas, there remains only a sparse growth of *Carex* spp., *Artemisia frigida*, *Antennaria* spp. This condition prevails in the overgrazed pasture shown in Figs. 3 and 4, an area adjoining a fescue meadow and tenanted originally by the *Festuca scabrella* association. Moreover, other species may come in, notably *Stipa comata*, *Bouteloua*

PLATE I



FIG. 3. Fescue hay meadow near Calgary, showing haying operations. Overgrazed pasture in foreground was formerly fescue prairie.

FIG. 4. Same meadow and pasture as in Fig. 3, showing *Festuca scabrella* in the meadow, and *Artemisia frigida* in the pasture.



*gracilis*, and *Agropyron Smithii*, species of the plant association that characterizes the light brown soil zone. Indeed these species are so common in parts of the general region as to leave the impression that they belong there. No doubt these species did occur in the fescue or black soil region under natural conditions, but only on very dry, south-facing slopes or certain kinds of clay flats. From these localized areas they have spread to overgrazed pastures. On the east side of the Porcupine Hills, for instance, *Bouteloua gracilis* has become a dominant plant in many areas originally covered by fescue. This remarkable behavior of blue grama grass should be further investigated. Also worthy of mention in this connection is *Andropogon scoparius*, which has a sporadic distribution from Calgary southward to Pincher, occurring chiefly on denuded slopes with poor soil and scant vegetation. No doubt this grass, like *Bouteloua*, has occupied certain localized areas within the fescue region for many centuries, but recently has spread somewhat to ground formerly occupied by the fescue association.

The influence of bison and other native grazing animals on the virgin prairie has been the subject of much speculation. Though *Festuca scabrella* has been called buffalo bunch grass because the buffalo (bison) were allegedly fond of it, there seems to be no evidence that this grass was excessively grazed by these animals. It is doubtful whether our fescue grassland was grazed by buffalo even to the extent now utilized by domestic animals on the best ranges of the region. Larson (4) contends that buffalo had much the same effect upon the plains grassland as is currently exerted by domestic grazing animals, which was to produce a short grass type of vegetation. Therefore, according to Larson, the short grass plain is not a "disclimax". Our studies point to the conclusion that the *Festuca scabrella* association is not only the climax grassland for our region but that this climax was, in the main, realized and maintained during the time when buffalo were transient members of the same biotic community. Had the fescue association been held in a "disclimax" condition by the buffalo, it would be difficult to account for the present distribution of the climax association. Historical records point to the conclusion that the plains buffalo did not range northward in large numbers as far as our region and that the wood buffalo remained farther north.

### Fescue Prairie and Other Vegetation

The relationship between the fescue association and the grassland of southeastern Alberta has been discussed briefly above. The grassland of the southeastern region has been variously named and described in earlier publications (1, 2, 5, 6). Suffice it to say here that we prefer to characterize this prairie in terms of the climax state rather than in terms of the prevailing condition, which we are inclined to believe is largely disclimax. Since *Stipa comata* is considered to be the climax species, the climax prairie may be called the *Stipa comata* association. Important grasses in the disclimax, as it occurs today in most parts of the region, are *Bouteloua gracilis*, *Koeleria cristata*, *Poa secunda*, and *Agropyron Smithii*. The latter species is also important as a

subclimax dominant, especially on clay flats. The broken line in Fig. 1 is believed to represent approximately the middle of the tension zone between the climax *Festuca* and *Stipa* associations. Through grazing and other disturbances associated with settlement of the country, this natural tension line has been shifted considerably to the north and west. There are, of course, outliers of each association in the general region of the other, for example, the fescue association in the Cypress Hills.

The question of equilibrium between prairie and associated forest is one of perennial interest. We have little to add to the earlier discussions (3, 5, 6) of this topic for our region, except to emphasize the fact that poplar, willow, and certain other woody plants are tending to invade the fescue prairie. Regardless of the effects of earlier climatic shifts, the trend at present, which seems also to have been the main trend for many decades or even centuries, is for the woody species to encroach upon the grassland. Counteracting this tendency are the effects of burning, mowing, and, to a limited degree, grazing. In many of the ranching areas, for example, the north Porcupine Hills, willows are a constant source of annoyance in the fescue hay meadows and are kept down only by regular mowing. Similar areas, where grazed rather than mowed, have large clumps of willow (chiefly *Salix Bebbiana*), as well as other woody plants. Grazing does actually retard the development of willows and poplars, the degree of damage depending on grazing intensity, browsing habits of the animals, and the local environmental conditions. Certain other woody species in the fescue grassland, notably the shrubby cinquefoil, seem to be definitely favored by grazing. Further investigation is needed to fully elucidate the ecology of woody plants in fescue prairie under range conditions. Incidentally, we are prepared to support the opinion expressed by Roe (7) and by Soper (8) that buffalo did relatively little damage to woodland growth. As Roe emphasizes, fire seems to have been much more effective than the buffalo in causing deforestation and in retarding the advance of trees and shrubs into the prairie.

Natural succession from fescue prairie to wooded vegetation was most clearly evident in the north Porcupine Hills and north of these Hills. Here the grassland is commonly invaded by willow followed by poplar or, through root-suckering, directly by poplar. Willow and poplar are followed in due course by white spruce (*Picea glauca*), or, at higher altitudes, by *Pinus contorta* var. *latifolia* and *Pseudotsuga Douglasii*. As suggested in an earlier paper (5), white spruce may be regarded as the theoretical climax for much of the fescue grassland or black soil region, though natural burning tends to prevent the realization of this climax.

The occurrence of isolated aspen poplar groves on moist slopes throughout our parkland region has not been satisfactorily explained (5). These groves may possibly be remnants of a continuous forest cover that prevailed during a cooler wetter period. On the other hand, it seems more likely that they started individually from seed, either long ago or in relatively recent times. Critical soil studies should shed some light on this problem.

Prairie patches or 'islands' in the wooded parts of our region are readily explained as relicts of a continuous grassland that covered the region during a former drier period. The 'islands' are found on dry slopes and on light sandy soils. One of these was observed near Beauvallon, east of Two Hills, where most of the natural vegetation for many miles around was wooded. This was a level area, with light soil and a covering of grasses, sedges, and forbs characteristic of the fescue association. Though only scattered wisps of *Festuca scabrella* were found, we are confident that this grass was abundant in the natural cover, and has been reduced by rather heavy pasturing of the area. Prairie patches on very dry slopes within the wooded region are usually somewhat denuded by overgrazing. Their characteristic flora of *Stipa* spp., *Bouteloua gracilis*, *Agropyron* spp., *Carex* spp., etc., resembles the *Stipa* association more closely than the *Festuca scabrella* association. Though the *Stipa* community has no doubt been favored on these dry slopes since the advent of white man, this type of vegetation was surely established there from early times and probably since a much drier climate prevailed in the region. This aspect of the vegetation therefore lends strong support to the view that our present climate was preceded by a long drier period.

Mention may be made of a few minor aspects of the fescue grassland in relation to other types of vegetation. In many localized areas, colonies (societies) of *Symphoricarpos occidentalis* occur in the prairie. These may have developed because of special edaphic conditions or because of disturbances by animals (5). Elsewhere there are thickets of *Rosa* spp. In parts of the foothills, various other shrubs, e.g. *Betula glandulosa*, *Juniperus horizontalis*, *Arctostaphylos uva-ursi*, tend to invade the fescue grassland. On lower ground, the fescue meets other shrubs, such as *Salix petiolaris* and *S. discolor*, and also lowland grasses, sedges, rushes, etc., including such grasses as *Deschampsia caespitosa*, *Calamagrostis canadensis*, and *Poa palustris*. Where the various woody and lowland communities meet the fescue grassland, the latter is, of course, not developed as the genuine fescue community but has an admixture of foreign species. Most of the species listed for the fescue grassland in the first column of Table II belong in this category.

### Grassland Vegetation and Soils

Our studies support views generally held by students of climate-soil-vegetation relationships in the steppe-woodland regions of Russia and North America, viz.

1. That black (chernozem) soils are essentially grassland soils developed through many centuries in a cool continental climate of moderate rainfall.
2. That the steppe or prairie areas, likewise the black soil areas, were originally larger than now, and have been invaded by forest rather recently in soil history.
3. That the black soils thus covered by forest have been modified by podsolization to become 'degraded chernozems' or transition (gray-black) soils.



Emphasizing here our conclusion that the natural vegetation of the black soil and gray-black soil zones through many centuries was a *Festuca scabrella* association in which a single species predominated, and assuming that most of the organic matter in prairie soils has derived directly or indirectly from the roots and crowns of grasses, we may conclude that the bulk of the organic substance in the black soils was produced by the dominant grass species, *Festuca scabrella*. Even in the dark brown soil zone, this grass seems to have flourished to some extent and no doubt has furnished that soil with much organic material. It appears therefore that a large proportion of the agricultural wealth represented by the black and related soils of our region had its origin in the activity of just one kind of higher plant.

### Utilization Problems

Though this investigation emphasizes the profound changes brought about in the fescue grassland through grazing and mowing, the ecology of the dominant grass, *Festuca scabrella*, is still not well understood. The relatively high crowns of this species seem to require for their vigorous growth the shelter of old leaves and the protection mutually afforded one another. Not only grazing and mowing, but also burning, may bring about desiccation of the fescue tussocks. The rôle of prairie fires in relation to utilization should be further investigated.

Questions regarding the regeneration of *Festuca scabrella* remain unanswered, for instance, (a) the time required for recovery of fescue grassland when given a rest period and following various stages of depletion, (b) factors affecting seed-setting and establishment of seedlings. The heading out of this grass seems to vary greatly from place to place and from season to season, but no correlation between heading and such variables as soil, exposure, composition of stand, or even vigor of plants has been established. It may be significant that the generally good heading of this species in western and central Alberta during the year 1947 followed a winter of exceptionally heavy snowfall.

There is also the question whether, in the interest of range stability, the dominance of the relatively high forage-producing and palatable *Festuca scabrella* should be maintained, or alternatively, whether this grass might well be replaced in large part by such species as *Agropyron trachycaulum*, *Stipa* spp., *Koeleria cristata*, *Danthonia Parryi*, and certain forbs. In cattle-raising regions the areas dominated by *Festuca scabrella* are prized as native hay lands. On the other hand, in certain sheep-ranching regions the disappearance of *Festuca scabrella* is looked upon with favor as the sheep seem to prefer the fine-leaved shorter grasses. In this connection, additional information is needed on the relative nutritive values of the secondary species and on their ability to withstand grazing. Further research should be done too on the successional changes induced by utilization and on the effects of various kinds of grassland cover on soil erosion initiated by water and wind.

Though much remains to be learned concerning the proper utilization of Alberta grasslands, investigations already carried out have provided valuable

information of a practical nature (1, 2). Mention may be made of the use of quantitative studies on ground cover to establish an index as to forage yield. From this information and a knowledge of succession phenomena, grazing lease rentals have been set with some degree of success.

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# AEROBIC BACTERIA THAT DECOMPOSE CELLULOSE, ISOLATED FROM QUEBEC SOILS

## I. ISOLATION AND DESCRIPTION OF THE SPECIES<sup>1</sup>

BY A. M. ALARIE<sup>2</sup> AND P. H. H. GRAY<sup>3</sup>

### Abstract

Soil bacteria that hydrolyze starch were isolated by means of an elective culture medium with this polysaccharide as the sole source of carbon; their ability to decompose cellulose was then tested. Thirteen strains of cellulose decomposing bacteria were thus isolated from several Quebec soils. The bacteria have been assigned to three recognized genera under eight new specific names. Their elevation to species rank is based on differentiations comparable to those used in Bergey's Manual. The following new species have been proposed: *Vibrio perimastix*, *Vibrio hyperion*, *Bacterium infirme*, *Bacillus vagans*, *Bacillus Kellermant*, *Bacillus soli*, *Bacillus torquens*, and *Bacillus effluens*.

### Introduction

Under natural conditions cellulose is biochemically one of the most important of the compounds that reach the soil as a component of plant residues. This is especially true of forest soils and pastures. In agricultural soils cellulose is added, along with other materials of significance in microbial nutrition, by the plowing-in of the roots of many annual crops, and by the addition of farmyard manure. In soils under crops the normal equilibrium between groups of micro-organisms is disturbed by the introduction of plant residues.

Recent work on the cellulolytic (cellulose decomposing) bacteria of soil has been reviewed by Norman and Fuller (20, pp. 239-264). These authors consider that the aerobic cellulose bacteria in soil are mostly versatile organisms that, individually, may not be very vigorous in attacking cellulose but that are likely to be able to maintain themselves in a heterogeneous soil population that is normally utilizing not cellulose alone in the pure state but a substrate of which cellulose is only one of many constituents. These authors developed cellulose-dextrin media that they claim favor the growth of cellulose decomposing bacteria of the genus *Sporocytophaga* (*Myxobacteriales*) as well as that of the versatile species. Since the majority of the versatile species of cellulolytic bacteria are also recorded as capable of using starch, and since this carbohydrate is less refractory to attack by micro-organisms, it was decided to use starch instead of cellulose as the source of carbon in a mineral-salts enrichment medium in an attempt to isolate cellulolytic bacteria from

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a variety of Quebec soils. The main reason for this departure from the usual procedure of inoculating soil into media containing filter paper was the difficulty that in the latter media growth of cellulolytic bacteria was usually slow and led to the development of many associated bacteria that interfered with an easy isolation of the organisms sought for.

### Summary of Recent Studies

By far the most important group of cellulose decomposing bacteria is represented by versatile organisms. They attack a wide range of carbon and nitrogen sources, though not necessarily vigorously. In a rapid survey of the literature on these bacteria we have eliminated reference to those that did not grow on starch as this carbohydrate is the basic compound of the enrichment and isolation media used in the present study. A nearly complete review of the literature, up to 1946, has been presented by Harmsen (12).

*Vibrio* species have been described by Gray and Chambers (8), Dubos (3), Kalnins (15), Itano and Arakawa (13), Stapp and Bortels (26), and Gray (7). Eighteen species in all showed diastatic activity. Five strains not named by Jensen (14) showed a similar aptitude.

Of the large group of *Cellulomonas* (26 species) that compose the studies of Rullmann (21), Kellerman and McBeth (16), Kellerman, McBeth, Scales, and Smith (17), McBeth and Scales (19), McBeth (18), Sack (22), and Sanborn and Hamilton (23), only one species, *Cellulomonas acidula* Kellerman *et al.* (17), did not grow on starch. Those of Rullmann and of Sack have not been fully described as regards their diastatic activity. The only *Cellulomonas* isolated from Quebec soils, by Hamilton (11), has not been thoroughly studied and cannot be catalogued here as no species name has been given.

Of the 10 species of *Bacillus* that have been reported in the studies of Kellerman and McBeth (16), McBeth and Scales (19), McBeth (18), Kalnins (15), Simola (24), Zarembka (27), and Fuller and Norman (4), one is given as not hydrolyzing starch, *Bacillus imminutus* McBeth (18). Two of Sack (22) are not sufficiently described. Jensen (14) also quoted two unidentified species as hydrolyzing starch.

The situation is similar with regard to the *Pseudomonas* group. Of the three described by Kellerman *et al.* (17), McBeth and Scales (19), and McBeth (18) and classified as *Pseudomonas* by Bergey *et al.*, 5th edition (2), and of the three given by Fuller and Norman (4), only the one of the latter authors, *P. erythra*, does not grow on starch, and seems to be an obligate cellulose and cellulose-dextrin decomposer.

Three species of unidentified *Corynebacterium* (Jensen, 14) are also starch hydrolyzers.

One species of what appears to be a *Vibrio* and called *Cytobacter polonicum* by Gutgisser (10) grew well on starch but the starch hydrolysis is not mentioned. The same applies to *Cellulococcus albus* as reported by Sack (22).

The three strains of *Bacterium cellaresolvens*  $\alpha$ ,  $\beta$ , and  $\gamma$  of Groenewege (9) and Jensen's (14) unidentified *Bacterium R* and *CO* are starch hydrolyzers. The same applies to *Achromobacter picrum* recently described by Fuller and Norman (4).

It should be noted that Bergey's Manual, 5th edition (2), reports under the genus *Cellulomonas* a number of species originally described as *Bacillus* or *Bacterium*; it should also be noted that many organisms mentioned above are not to be found in the Manual.

This concludes a general review of the versatile soil bacteria reported to decompose cellulose. As to rapidity of action, the literature suggests that the many versatile bacteria are not less active than the other forms. Both groups are widely distributed in soil and have been reported throughout the world. The diastatic power is a property of the majority of the versatile organisms above, and with this in mind, the authors have undertaken the present study.

Cellulose decomposing bacteria have been reported only on very rare occasions from Quebec soils. Hamilton (11) has described an organism active in cellulose decomposition but did not go further than the genus for the purpose of his study; the organism was a *Cellulomonas*, showing only few differences from *Pseudomonas subcreta* McBeth and Scales (19). (Bergey *et al.*, 1923, 1st edition, emended the name to *Cellulomonas subcreta*, but gives *Pseudomonas* in the 5th edition, 1939). Gray (6) investigated the microflora of separate horizons of podsol soils; he ascertained the presence of *Cytophaga Hutchinsonii* (now *Sporocytophaga myxococcoides* Stanier), and of other forms not isolated. In a more recent study Gray (7) described *Vibrio amylocella* that had been isolated from a manured garden soil.

## Experimental

### METHODS

#### *Soils and Methods of Sampling*

Thirteen soil samples were taken at different places on the farm at MacDonald College, and from soils in different localities in the adjacent counties of Soulange and Vaudreuil, with no particular regard to the soil type or crops.

To prepare the profile for sampling, the trowel or shovel was cleaned with paper and rags, washed, and flamed with alcohol at each new profile. Samples were generally taken at from three to five inches from the top of the profile and special mention is made when this was not the case. One sample was taken of each profile. Samples were collected in small glass vials (15  $\times$  75 mm.) previously plugged with cotton and sterilized. At each soil profile, the requisite number of vials were forced horizontally, with a slight screwing motion, into undercut and well scraped profiles in order to avoid contamination from surface dust. The bottles were then closed with sterile rubber stoppers, wrapped, and taken to the laboratory.

*Isolation of Starch and Cellulose Hydrolyzing Bacteria*

In order to obtain a dense growth of powerful starch hydrolyzing organisms, elective cultures were set up by inoculating 50 ml. of a starch\* solution in 250 ml. Erlenmeyer flasks with approximately 1 gm. of fresh soil. The medium contained starch 0.2%, ammonium chloride 0.2%. The mineral salt solution used throughout the course of the work was composed as follows (Kalnins, 15):

	%
Potassium monohydrogen phosphate	0.1
Magnesium sulphate	0.02
Sodium chloride	0.01
Calcium chloride	0.01
Ferric chloride	0.002

After 24 hr. of growth at 28° C., the cultures were plated with starch agar, containing peptone instead of ammonium chloride, with the same mineral salts. The dilutions were made with a 3 mm. loop, transferring a loopful of the elective culture to a tube of melted agar and again a loopful from the latter to a second tube. Three such dilutions were made and that in the last tube in duplicate. The plates prepared from the tubes were incubated at 28° C. After three to five days, those colonies that seemed different were transferred to grid plates of starch agar. The underside of a starch agar plate was divided by means of wax-pencil marks into 16 squares of even dimensions and marked for orientation. A sheet of paper marked in the same fashion and numbered from 1 to 16, placed under the plate, permitted the localization of the different inoculations without turning the plate upside down at each inoculation. This arrangement insured a rapid means of identification of the different colonies. The grid plates were incubated for 48 hr. at 28° C. and then all the colonies that developed were transferred to slants of starch agar of the same composition as that in the plates. After making sure that all the organisms had grown on the slants the grid plates were flooded with an iodine solution and the organisms that hydrolyzed the starch were kept for tests on cellulose. Most of the organisms isolated in this manner were pure cultures.

The ability of these bacteria to decompose cellulose was ascertained in a medium containing filter paper as described below. A comparison of the nutrient value of different sources of nitrogen for these bacteria forms the subject matter of another paper.

*Culture Media and Cultural Methods*

Since many of the strains isolated proved to grow very poorly in the presence of meat extract or of nitrate salts, the culture media used throughout the tests were supplied with nitrogen in the form of peptone. The basic mineral salt solution for most of the media was that used by Kalnins (15). The different salts were kept in separate stock solutions at 100-fold concentrations. A brief description of the different culture media used is given below.

\* This was BDH AnalaR. It was incorporated into media in the manner described by the junior author (7).

### *Starch Broth and Agar*

Starch was mixed with 25 to 30 ml. of cold distilled water, and then poured into boiling water to dissolve. The solution was boiled for approximately one minute or until opalescent; 0.2% peptone and the mineral salt solution were then added and the medium brought to final volume.

In the case of starch agar, 1.5% of agar was added to the starch broth.

### *Cellulose Salts Liquid Medium*

The nutrient solution was composed of 0.2% peptone and the required quantity of mineral salts in distilled water. The bacteria were cultured in tubes containing 10 ml. quantities with a strip of filter paper (Whatman No. 41) of such a length as to emerge over the surface of the liquid.

### *Cellulose Agar*

This culture medium consisted of finely precipitated cellulose incorporated into a peptone mineral salt agar.

Six grams of filter paper were precipitated with sulphuric acid, according to the method given by Kalnins (15). Finely cut filter paper was dissolved for 20 sec. in slightly diluted sulphuric acid (100 ml. of concentrated sulphuric acid, sp. gr. 1.81, in 60 ml. of distilled water). Cellulose was reprecipitated in bringing the volume rapidly up to two litres with water and allowed to settle overnight in 10 to 15 litres of water. The supernatant liquid was siphoned off and the precipitate filtered in a large Buchner funnel with repeated washing until all traces of acid were removed. The cellulose mat obtained on filtration was resuspended in 800 ml. of water for storage; a very even precipitate was obtained by agitation with an egg beater. This gave an approximate concentration of 0.4% cellulose, which proved to be a convenient density of fibres in the agar plates.

The agar concentration was lowered to 0.8% as advised for the studies of the *Cytophaga* group (Stapp and Bortels, 26, Stanier, 25); it proved to be very valuable in these studies as it gave better evidence of enzymic action and larger colonies.

### *Gelatin*

Two formulae for gelatin were used. The common nutrient gelatin, with 120 gm. of gelatin, was first used, but as some strains were inhibited by the presence of meat extract, it was decided to test for gelatin liquefaction in a medium containing gelatin 10%, starch 0.2%, peptone 0.2%, and the mineral salt solution.

### *Litmus Milk*

The dehydrated medium of the Difco Laboratories were used.

### *Nutrient Broth and Agar*

The ordinary nutrient broth was composed of meat extract 0.3%, and peptone 0.5%. Agar 1.5% was added to nutrient broth in order to obtain the nutrient agar.

*Dextrose Agar*

This culture medium was composed of dextrose 0.5%, peptone 0.2%, and the mineral salt solution.

*Carbohydrate Broths*

The different mono- and polysaccharides, alcohols, and glucosides as listed in Table I were added as 0.5% to the basal mineral salt solution with peptone 0.2%. Phenol red in 0.024% concentration was used as indicator.

Sterilization under pressure in the autoclave caused very little change in the pH, and it was assumed that the more sensitive sugars, such as glucose, were not hydrolyzed in presence of the mineral salts at a pH value of about 8.0.

*Medium for Ammonia Production*

The test was carried out on two-month-old cultures in cellulose solution, and on three- and six-day-old cultures in urea broth containing urea 2% and 'yeastrel' 0.3%.

*Medium for Nitrate Reduction*

Nitrite formation from nitrate was tested in three- and six-day-old cultures in starch mineral salt broth containing sodium nitrate 0.2%.

*Medium for  $H_2S$  Production*

Kligler iron agar produced by the Difco Laboratories was used to test for the production of  $H_2S$ .

*Medium for Indol Production*

The culture medium used for this was a Difco product, Bacto-troptone. The test for indol was made in six-day-old cultures by means of Ehrlich's reagent; 0.5 ml. of the reagent was run into slanting tubes of the culture so that it remained on top of the liquid.

*Relation to Free Oxygen*

The anaerobic faculty of the strains under observation was studied in 300 ml. bottles with 30 ml. of pyrogallol dissolved in 20% potassium hydroxide. A tube of reduced methylene blue was placed with the cultures in each bottle and the bottles were tightly closed with rubber stoppers. After eight days' incubation at 28° C., the bottles were opened for observation and all the tubes with methylene blue were still colorless. After exposure to atmospheric oxygen they all turned blue within a few minutes.

*Staining Methods**Cell Morphology*

Form and dimensions of the cells were studied on 48-hr.-old (occasionally 24 hr.) starch agar slants. The films were fixed by flame and stained with aqueous crystal violet.

*Spores and Spore Staining*

Spore location, form, and dimensions were determined with starch agar cultures of different ages because of the different ability of the strains to form



spores more or less rapidly. One strain, *Bacillus soli*, had to be studied on nutrient agar because it produced only a few spores on starch agar.

The Ziehl-Neelsen method for acid-fast organisms was used throughout for staining spores as it affords a counterstain, methylene blue, for the cell itself. Pretreatment for 30 sec. with chromic acid, 0.5%, proved to increase the receptivity of the stain by the spores.

### *Gram Stain*

Hucker's modification of Gram's method was used with Atkins' sodium hydroxide-iodine solution. The stain was used with 24-hr.-old cultures on starch agar slants and repeated after eight days of growth. A 24-hr.-old culture of *B. subtilis* was used as a control.

### *Flagella Stain*

The bacteria from a 24-hr.-old culture on starch agar slant were allowed to swarm in weak suspension, in a few drops of distilled water, for 15 min. A loopful of the suspension was then placed on a well roasted and cooled slide; from this loopful a smaller one was transferred to an adjacent loopful of distilled water. By this means many films were prepared at once on one slide. Gray's (5) method of staining flagella was used. Conventional terminology has been adopted to indicate the apparent placement of these appendages.

## Results

### *Starch Hydrolyzing Bacteria*

One hundred and three strains were isolated. Many of the organisms were alike on the basis of their colony formation and morphology.

Most of the cultures could be assigned to the genus *Bacillus*, some to *Bacterium*. A few *Pseudomonas*, two strains of *Proactinomyces*, and one *Micrococcus* were also isolated.

Only 13 strains proved to be cellulose hydrolyzers as well. These only were studied more in detail.

### *Cellulose and Starch Hydrolyzing Bacteria*

Six only of the 13 soils examined yielded 13 strains of the bacteria, which are numbered and described below under their specific names.

The eight species are assigned to three genera, namely, five *Bacillus*, two *Vibrio*, and one *Bacterium*. The latter genus has been adopted in preference to *Cellulomonas* as advocated by Bergey *et al.* (2), since it is believed that generic differentiation should not be based solely upon this one biochemical activity.

The organisms are described below in respect of morphology and characters on nutrient media, a summary of all characters that would admit of tabular treatment are shown in Table I. Characters common to all of the strains are as follows: all are able to grow anaerobically and are motile, Gram-negative, and do not produce indol or hydrogen sulphide; only *V. perimastix* and

*B. effluens* reduce nitrate to nitrite; and traces of ammonia were found in old cellulose cultures of *B. vagans* and *B. Kellermani*; none produced ammonia from urea.

From the table of biochemical characteristics it might be held that the species numbered 4 and 5 should be considered as varieties of one species, the sole difference between them being that the strains of *B. Kellermani* (No. 5) did not decompose dulcitol; reference to their cultural characters on agar would, however, suggest that they differ sufficiently; they are therefore tentatively proposed as separate species.

Photographic reproductions of camera lucida drawings of cell morphology, flagella, and spores, and of growth upon agar plates, appeared as plates in the senior author's thesis (Alarie, 1).

*Species 1—Vibrio perimastix* n. sp.

(The specific name is derived from the Greek, *peri*, around, and *mastix*, whip)

*Cell form*: curved rods,  $0.4-0.7\mu$  by  $1.4-5.6\mu$  with 8 to 20 peritrichous flagella.

*Agar colony*: spreading, irregular, flat, bluish (barely visible), butyrous; 8 to 12 mm. in 24 hr.; similar on starch and dextrose agars.

*Agar slant*: thin, colorless to grayish, curly, barely visible, butyrous.

*Nutrient broth*: no visible growth.

*Gelatin stab*: no liquefaction.

*Potato*: good filiform growth, light yellow, shining.

*Litmus milk*: no change.

*Cellulose decomposition*: with peptone, paper broken in three or five days; with nitrate in eight or nine days; on cellulose agar the growth is spreading and the hydrolysis is superficial.

*Source*: isolated from a muck soil.

*Species 2—Vibrio hyperion* n.sp.

(Specific name from the Greek, Hyperion, the sun-god; in allusion to the flame-shaped projections of the colonies)

*Cell form*: short curved rods,  $0.4-0.7\mu$  by  $1.4-2.4\mu$ ; a few cells  $4.2\mu$  long; with one polar flagellum.

*Agar colony*: on starch agar, spreading, with curly projections, irregularly raised, shining, bluish gray, butyrous; curling less on dextrose agar, and colonies yellowish gray.

*Agar slant*: filiform, with some curling; shining, yellowish gray, semiopaque; old cultures yellow.

*Nutrient broth*: no visible growth.

*Gelatin stab*: no liquefaction.

*Potato*: good filiform growth, buff, shining.

*Litmus milk*: no change.

*Cellulose decomposition:* with peptone, paper broken in five or seven days, and tinged with yellow; liquid yellow in old cultures; with nitrate, paper broken after three weeks or longer. Well defined hydrolysis on cellulose agar.

*Source:* from an unfertilized soil under pasture.

*Species 3—Bacterium infirme* n.sp.

(Specific name from the Latin, infirmus, weak)

This organism was not viable after some months.

*Cell form:* straight rods; a few curved;  $0.5-0.7\mu$  by  $1.7-2.8\mu$ , some up to  $6.3\mu$ ; single or paired; with 5 to 15 peritrichous flagella.

*Agar colony:* 3 to 4 mm., white, circular, umbonate to convex, smooth, translucent, butyrous.

*Agar slant:* filiform, grayish, translucent, butyrous; occasionally with gas in the butt.

*Nutrient broth:* turbid, with white slimy sediment.

*Gelatin stab:* no liquefaction; gas with starch-peptone.

*Potato:* filiform, scanty, buff, shining, dull white border.

*Litmus milk:* acid.

*Cellulose:* with peptone, paper broken in 17 to 19 days; with nitrate, in 30 days; on agar, decomposition not visible.

*Source:* muck soil, with *V. perimastix*.

*Species 4—Bacillus vagans* n.sp.

(The specific name is Latin, wandering, in allusion to the spreading growth on cellulose agar)

*Cell form:* straight rods,  $0.7-0.8\mu$  by  $1.4-4.2\mu$ , single or paired; with 5 to 15 peritrichous flagella; spores central to subcentral; slightly broader than the cell.

*Agar colony:* 2 to 3 mm. circular, slightly convex, bluish, shining, translucent, butyrous.

*Agar slant:* filiform, grayish, shining, translucent, gummy.

*Nutrient broth:* slightly turbid; a scanty viscid sediment.

*Gelatin stab:* funnel-shaped liquefaction.

*Potato:* filiform, light yellow, shining.

*Litmus milk:* slightly acid.

*Cellulose:* paper broken in 8 to 10 days; spreading colonies on agar decomposed cellulose without clear enzymic zones.

*Source:* two strains from the organic matter layer of a podsol soil under forest cover.

*Species 5—Bacillus Kellermani* n.sp.

(Specific name, in honour of Dr. K. F. Kellerman)

*Cell form*: straight rods, with squared ends,  $0.7-0.8\mu$  by  $1.0-3.5\mu$ , single or paired; flagella 5 to 15, peritrichous; spores central to sub-central, slightly broader than the cell.

*Agar colony*: 2.5 to 3.5 mm., nearly circular, white, umbilicate, shining, gummy.

*Agar slant*: filiform, cream-colored or white, shining, opaque, gummy.

*Nutrient broth*: slightly turbid, white sediment.

*Gelatin stab*: stratiform liquefaction.

*Potato*: filiform, white, dull.

*Litmus milk*: slightly acid.

*Cellulose*: with peptone, paper broken in seven days; with nitrate, in 16 or 21 days; decomposition on agar not visible.

*Source*: two strains were isolated, from two different pasture soils that had not been fertilized.

*Species 6—Bacillus soli* n.sp.

(The specific name is Latin, *solum*, the soil)

*Cell form*: straight, a few curved rods;  $0.7-0.8\mu$  by  $1.4-3.5\mu$ , single cells, with 4 to 12 peritrichous flagella; spores terminal.

*Agar colony*: 2 to 5 mm., nearly circular, grayish to white, convex, shining, translucent, butyrous.

*Agar slant*: filiform, grayish, shining, translucent; gas in the butt.

*Nutrient broth*: turbid, white slimy sediment.

*Gelatin stab*: no liquefaction; with starch the gel is broken by gas.

*Potato*: filiform, buff, shining, with dull white border.

*Litmus milk*: litmus reduced.

*Cellulose*: with peptone, paper broken in 21 days; with nitrate in 30 days; no enzymic clear zone on agar.

*Source*: from a field under oats that had not been fertilized.

*Species 7—Bacillus torquens* n.sp.

(Specific name from Latin, *torquere*, to twist, in allusion to the manner of growth on agar plates)

*Cell form*: short straight and curved rods,  $0.5-0.7\mu$  by  $1.4-2.1\mu$ , with 4 to 16 peritrichous flagella; spores terminal or subterminal, centrally placed and arising from a 'sporocyst' in clostridium cells formed on starch; these latter stain deeply at one end, and measure  $0.8-1.4\mu$  by  $2.1-4.9\mu$ .

*Agar colony*: motile by threadlike projections up to 25 mm. long, where new colonies form; grayish, shining, butyrous.

*Agar slant*: filiform, with some projections, yellowish gray, shining, semi-opaque, butyrous, later slimy and agar tinged with yellow.

*Nutrient broth*: no visible growth.

*Gelatin stab*: no liquefaction.

*Potato*: filiform, buff, shining.

*Litmus milk*: no change.

*Cellulose*: with peptone, paper broken in four to seven days; with nitrate, no decomposition; the paper and, later, the fluid tinged with yellow; a well defined enzymic clear zone on agar, the fibres being completely decomposed.

*Sources*: three strains from a field of potatoes that had been fertilized.

*Species 8*—*Bacillus effluens* n.sp.

(Specific name from Latin, *effluere*, to flow outwards, in allusion to the swarming projections from the colonies)

*Cell form*: curved rods,  $0.5-0.8\mu$  by  $1.4-4.2\mu$ , single; on starch agar developing to clostridia  $0.9-1.4\mu$  by  $2.8-5.6\mu$  staining deeply at one end; with 5 to 16 peritrichous flagella; spores terminal or subterminal in both forms of cell; no sporocysts.

*Agar colony*: with *starch*, 1 to 4 mm. circular, convex, white, shining, gummy, occasionally swarming; with *dextrose*, yellowish white, extensive swarming by one strain, less with the other, projections up to 25 mm.

*Agar slant*: beaded to filiform, margin sometimes with curling projections, gray, tinged with yellow; when older, shining, opaque, gummy, firmly adherent.

*Nutrient broth*: no visible growth.

*Gelatin stab*: no liquefaction.

*Potato*: scanty growth, light yellow, shining.

*Litmus milk*: no change.

*Cellulose*: with peptone, paper broken in 7 to 10 days; with nitrate in 14 days; growth spreading on agar, without a clear zone.

*Source*: two strains from a field under potatoes that had been fertilized.

### Conclusions

From the results of this study, it is evident that starch is well suited for the isolation of cellulose decomposing bacteria. If not all cultures were pure at the first isolation, it was relatively easy to purify them by means of dilution followed by a second plating. Difficulties were met with only in the purification of *B. torquens* as it was thought that the slimy cultures would carry in the dilution some cells of *V. hyperion* to which it is morphologically very similar in young cultures, and in physiological characteristics when older. Plating on starch agar of dilutions in distilled water after treatment for spores



(10 to 12 min. heating at 80° C.) finally gave a pure culture. No other difficulties were met with in the isolations.

As was expected, no specialized cellulose decomposing organisms were found, but the organisms isolated exhibit a great variety in their activities, some breaking the filter paper strip in mineral salt solution in three or seven days, as was the case with *V. perimastix*, and *B. torquens*, while some others, e.g., *Bacterium infirme* and *Bacillus soli*, developed that far only after three weeks. The fact also that the organisms isolated on starch and described as cellulose decomposers grow on many carbohydrates indicates that the versatile cellulose-hydrolyzing microflora is well distributed.

It may be said against the use of starch in the isolation of cellulose decomposing bacteria that the scheme of isolation is long and furthermore that keeping the stock cultures on starch will cause the organisms to loose their ability to decompose cellulose. To the first objection it may be stated that the procedure is not longer than any other since pure cultures are very readily obtained. But what is likely to happen, in the elective cultures, is that the active cellulose decomposing bacteria, having a weaker diastatic power, may be ruled out in numbers by the strongly diastatic bacteria of weaker activity in the cellulose breakdown. This may well be the limiting condition of this method. As to the loss of activity of the bacteria when incessantly grown on starch, Simola (24) attributed this phenomenon to the fact that mineral and not organic nitrogen was fed to the bacteria. The organisms described in the present work have been grown on starch peptone mineral salt agar slants and their ability to retain their cellulolytic ability was confirmed in studies made some months later.

It may also be advocated in favor of the use of starch that its composition is more uniform than that of the fractions obtained by the hydrolysis of cellulose by strong acids.

The *Bacillus* group is reported officially here for the first time for Quebec soils, though some previous unpublished investigations by the junior author had shown that species of this genus capable of decomposing cellulose were present; the present work, indeed, has been based upon those findings.

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## AN INDICATOR AGAR FOR THE DETERMINATION OF THE RELATIVE CONCENTRATION OF ASCORBIC ACID IN POTATO TUBER TISSUE<sup>1</sup>

BY WM. NEWTON<sup>2</sup>

### Abstract

An indicator agar that reveals the approximate concentration of ascorbic acid in potato tuber tissue by direct contact is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10% solution of potassium iodide, and 5 ml. of a 1% solution of soluble starch. After cooling to 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.02 *N* solution of potassium iodate are added and the mix is immediately poured into Petri dishes. When uniform filter paper discs saturated with standard ascorbic acid solutions or uniform tuber tissue discs are placed upon the agar surface, after 24 hr. at 5° C., the size of the area decolorized is directly related to the concentration of ascorbic acid in the solutions and tissue discs. Tissue discs from tubers affected with mosaic and leaf roll decolorize a greater area than discs from normal tubers from the same variety, and the decolorization is independent of tuber size. The diagnostic accuracy of the method is further strengthened by the tendency of virus infected tissue to remain white and healthy tissue to become blue-black.

### Introduction

Indicator agars were studied in the hope of developing a simple means of utilizing the ascorbic contents of plant tissue as indices of virus disease infection. Smith and Paterson (2) have shown that potato tubers infected with mosaic and leaf roll contain higher contents of ascorbic acid than tubers from healthy plants of the same variety, as judged by the titration values of trichloroacetic acid extracts with the dye 2,6-dichlorophenolindophenol. These authors have shown also that within the same variety the variation in ascorbic acid between tubers is relatively small and appears to be independent of the size of the tubers and the manuring of the crop. On the other hand, they have shown that there is a loss of ascorbic acid during storage, and the loss probably varies with the storage environment. A preliminary study of three potato varieties grown in British Columbia, viz., Netted Gem, Burbank, and White Rose, has confirmed the conclusion of these authors that within a single variety an abnormally high content of ascorbic acid in the tuber tissue is a reliable index of virus infection. Simplification of the means of determining relative concentrations appeared possible when it was discovered that, if uniform tuber tissue discs are placed upon agar surfaces, the ascorbic acid diffuses into the agar in amounts roughly proportional to the concentration within the tissue.

### The Indicator Agar Method

The indicator agar is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10% solution of potassium iodide, and 5 ml. of a 1% solution of

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soluble starch. When the agar dissolves and the mix is cooled to approximately 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.02 *N* solution of potassium iodate are added, and the indicator agar is immediately poured into Petri dishes, 10 ml. per 9 cm. dish, or proportionately greater amounts when larger dishes are used. Care must be taken that the depth of the agar is uniform. Some Petri dishes are unsuitable because the bottoms are not flat. The cooling of the agar solution prior to the addition of potassium iodate is essential. If the iodate is added at a temperature close to the boiling point, the iodine that is formed by the reaction of the constituents is lost, and the characteristic starch-iodine blue color of the indicator agar will not form. The Petri dishes are stored in a refrigerator until used, as it is assumed that storage at low temperatures preserves the sensitivity of the agar.

The sensitivity of the agar is measured by placing filter paper discs saturated with ascorbic acid solutions on the agar surface. The diameter of the decolorized zone, the halo around the discs, is a function of ascorbic acid concentration, the depth of the agar layer, and the concentration of the potassium iodate; consequently, if the depth of the agar is constant and the loss of iodine is negligible in the preparation of the indicator agar, the diameters of the halos are the same after 24 hr. at 5° C. when the filter paper discs are saturated with a standard ascorbic acid solution and are placed upon the indicator agar surfaces.

Tissue cylinders are removed from the potato tubers by means of a cork borer and discs approximately 5 mm. in thickness are cut therefrom by means of a razor blade. These are placed immediately on the surface of the indicator agar and the dishes are returned to the refrigerator. Discs from healthy tubers are placed in the same dishes with those from the corresponding variety infected with mosaic or leaf roll. The diameters of the halos are noted after 24 hr. at 5° C. The tests are carried out at 5° C. chiefly because of convenience. At this temperature the decolorization of the agar is slowed down sufficiently to avoid error through the practical difficulty of timing correctly the period that the discs are in contact with the agar. However, the conduct of the tests at higher temperatures has the advantage of speed. At 25° C. within two hours the halos surrounding discs from tubers affected with mosaic or leaf roll are appreciably larger than those around the discs from healthy tubers of the same variety.

## Results

When uniform tuber tissue discs of the potato varieties Netted Gem, Burbank, and White Rose were placed upon the indicator agar, after 24 hr. the halos were invariably larger around the discs derived from plants infected with leaf roll or mosaic than from healthy plants of the same variety. Typical examples are illustrated in Fig. 1, A to D.

In addition to the contrast in halo size, the upper surfaces of the discs derived from virus infected plants tended to remain wholly or partially white, while those from the tubers of healthy plants became blue-black. The

contrast in color and halo size is shown in Fig. 1, A. The blue-black color of the discs from healthy plants is probably caused by the volatile iodine.

Tissue from tubers infected with leaf roll apparently contains less ascorbic acid when derived from old than from new tubers of the same variety. The contrast is illustrated in Fig. 1, E, and represents the effect of discs from the 1946 and 1947 tuber crops.

The halo diameters as induced by discs from healthy tubers were approximately the same regardless of tuber size within a single variety (Fig. 1, F).

The indicator agar served well as a rapid method of determining the approximate concentration of ascorbic acid in miscellaneous fluids. The concentration in fluids was determined by comparing the size of the decolorized zones induced by single drops of standard ascorbic acid solutions and the unknowns, or by uniform filter paper discs saturated with the standards (Fig. 1, G) and the unknowns. The effect of adding a single drop of a standard ascorbic acid solution containing 50 mgm. per 100 ml. compared with a single drop of lemon juice is shown in Fig. 1, H. The lemon juice contained approximately 50 mgm. per 100 ml. as judged by the similar diameters of the decolorized areas.

Permanent records are conveniently obtained in the form of negative prints by placing the dishes in a darkroom on photographic paper, followed by exposure to light. The illustrations in Fig. 1 were obtained in this manner, with the exception of A and H, which were photographed in the ordinary manner to illustrate the typical blue-black color acquired by potato discs from healthy tubers in contrast with virus infected, and the influence of lemon juice in contrast with a standard ascorbic acid solution.

## Discussion

The preparation of the indicator agar was based upon an iodometric method for the chemical determination of ascorbic acid in plant tissue (1). Iodometric methods are not so popular as those based upon the reduction by ascorbic acid of the dye 2,6-dichlorophenolindophenol. Agars were prepared that contained this dye at a wide range of concentrations and as adjusted to a wide range of hydrogen ion concentrations, but none revealed the relative concentrations of ascorbic acid in potato tuber tissue discs so satisfactorily as the iodometric indicator agar described in this paper. At low concentrations the fading of the dye made it difficult to distinguish the decolorized area from the non-reduced agar zone. At higher concentrations the dye diffused back into the decolorized zone after it had reached its maximum size through the exhaustion of the ascorbic acid by oxidation. With the iodometric indicator agar, the decolorized zones remained constant after they had reached their maximum size through the exhaustion of the ascorbic acid.

The indicator agar is particularly useful in the diagnosis of leaf roll. Under conditions of drought, potato inspectors frequently have difficulty in distinguishing healthy plants from those infected with leaf roll, owing to the tendency of the leaves of healthy plants to curl when they lack moisture.

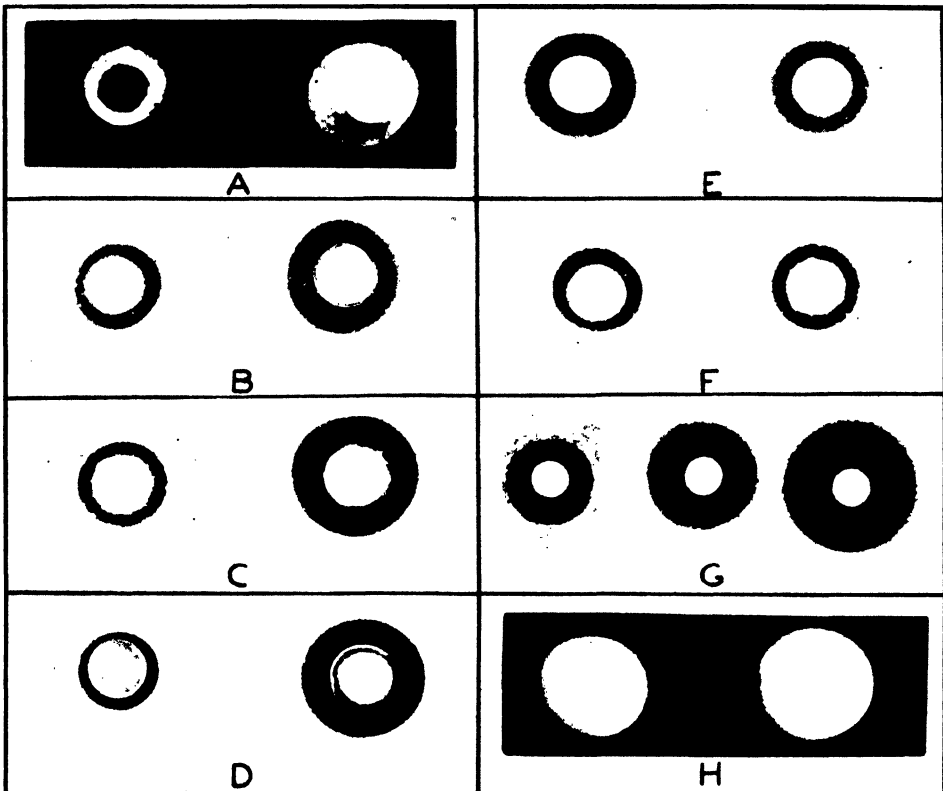


FIG. 1. The decolorization of indicator agar by tissue discs cut from potato tubers and by ascorbic acid solutions. A. Tuber discs from variety Burbank—left, healthy; right, affected by leaf roll. B. From variety Burbank left, healthy; right, affected by mosaic. C. From variety White Rose—left, healthy; right, affected by leaf roll. D. From variety White Rose—left, healthy; right, affected by mosaic. E. From variety Netted Gem affected by leaf roll—left, 1947 tuber; right, 1946 tuber. F. From variety Netted Gem left, healthy 2 cm. tuber; right, healthy 10 cm. tuber. G. Filter paper discs saturated with standard ascorbic acid solutions—left, 25 mgm. per 100 ml.; centre, 50 mgm. per 100 ml.; right, 100 mgm. per 100 ml. H. Left, one drop lemon juice; right, one drop standard ascorbic acid solution, 50 mgm. per 100 ml.



Although no difficulty has been experienced in distinguishing leaf roll or mosaic in tubers derived from infected planting stock, no evidence has been obtained that virus infection can be detected by the ascorbic acid content of the tubers when the infection has occurred late in the growing season.

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## STUDIES ON THE DECOMPOSITION OF CELLULOSE BY MICRO-ORGANISMS<sup>1</sup>

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### Abstract

The metabolism of an impure culture of an aerobic cellulose decomposing bacterium, *Vibrio perimastix*, was studied. The products of cellulose decomposition included carbon dioxide, a pigment resembling riboflavin, a bacterial polysaccharide, and traces of acid. Carbon dioxide was found to be essential for the decomposition of cellulose and could not be replaced by calcium carbonate. Increasing the carbon dioxide content of the air above 1.2% retarded growth on a glucose medium.

Respiration studies were carried out using cellulose, glucose, and cellobiose as substrates, and the possible role of the latter two as intermediates in cellulose decomposition was investigated. Glucose was produced from cellulose when toluene was added to cultures during active decomposition; evidence is presented that a dialyzable factor produced by the bacteria is essential for glucose formation. Phosphorylation inhibitors prevented growth of the bacteria on cellulose, glucose, and cellobiose, inhibited respiration of active cellulose cultures, and retarded the production of glucose by toluene-treated cultures. Cellulose treated with alkali to increase the proportion of amorphous to crystalline cellulose was more rapidly decomposed than untreated cellulose.

### Introduction

Cellulose is decomposed to simple chemical compounds by micro-organisms in a variety of natural processes. In this manner, a large part of the carbon of decaying vegetation is reconverted into forms utilizable by other living organisms, and a proper balance of the carbon cycle is maintained. However, very little is known about the micro-organisms involved or the mechanism by which the decomposition is accomplished.

This paper reports a study of the decomposition of cellulose by *Vibrio perimastix* (1), associated with an unidentified bacterium. The primary objective of this investigation was to obtain information regarding the enzymes active in the decomposition of cellulose by aerobic, mesophilic bacteria.

### I. Media

#### Salt Solution

The salt solution used was similar to that of Kalnins (5), and consisted of: 0.5 gm. disodium phosphate; 0.5 gm. monopotassium phosphate; 0.2 gm. magnesium sulphate septahydrate; 0.1 gm. calcium chloride dihydrate; 0.1 gm. sodium chloride; 0.02 gm. ferric chloride, and 1.0 gm. sodium nitrate

### Materials and Methods

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per litre of distilled water. The hydrogen ion concentration was approximately pH 6.6. When other hydrogen ion concentrations were desired the amounts of the individual phosphate salts were varied accordingly, but the total phosphate was always 0.1%.

#### *Cellulose*

Whatman No. 41 filter paper was used as the cellulose substrate.

#### *Cellulose Broth*

This consisted of 1 gm. of cellulose per 100 ml. salt solution.

#### *Cellulose Agar*

Prepared according to Kalnins (5).

#### *Starch, Glucose, and Cellobiose Broths*

These consisted of 1 gm. of the appropriate carbohydrate per 100 ml. salt solution. In the case of the latter two, the sugars were autoclaved in distilled water and sterilized salts then added.

#### *Starch-Peptide Agar*

Prepared by adding 1.5% washed agar and 1% peptone to starch broth.

### *II. The Culture*

The culture appeared to contain only one organism, also a *Vibrio*, in association with the *V. perimastix*. The impure culture was used in preference to a pure culture of *V. perimastix* since it was considerably more active in decomposing cellulose, and at the same time gave reproducible results. The bacteria were isolated from the soil (1).

Cellulose was actively decomposed in cellulose broth over a temperature range of 20° C. to 40° C. and at hydrogen ion concentrations from pH 5.0 to pH 7.5. The decomposition was accompanied by the production of a greenish-yellow pigment, the reduction of nitrate to nitrite, and a marked rise in the pH of the culture medium.

On starch-peptide agar the organisms produced a heavy, slimy growth, and the starch was partially hydrolyzed. This medium was used to maintain stock cultures, transfers being made periodically from cellulose medium. Inoculations from such cultures were as effective in attacking cellulose as those from cellulose broth. Heavy growth was also obtained with starch and glucose broths. There was only limited activity on cellulose agar, the clear zonations surrounding the colonies being very narrow.

### *III. Procedure*

Cellulose broth cultures of the organisms, contained in 1500 to 2000 ml. Erlenmeyer flasks, were aerated on a rotary shaker at 26.6° C. The initial hydrogen ion concentration was varied from pH 6.2 to 6.7.

After fermentation for 10 to 15 days the culture was filtered through a Whatman No. 42 filter paper. The residue was washed with hot water, alcohol, and ether, and dried to constant weight at 80° C.



The filtrate was tested for reducing sugars with Fehling's solution, and for pentoses or uronides by the phloroglucinol reaction. Ether extraction was used for the detection of acids, and steam distillation for alcohol. To precipitate mucilaginous materials, a portion of the filtrate was treated with acidified alcohol, according to Walker and Warren (14), or with dilute hydrochloric acid, according to Fåhræus (4).

### Experimental Results

#### I. Products of Cellulose Decomposition

Reducing sugars were not found in any of a large number of cultures examined. A positive phloroglucinol reaction was always given; the original filter paper gave a negative test. Acid distillation into phloroglucinol and hydrochloric acid gave a weak test for furfural, suggesting the possible presence of uronic acids. Traces of acid were detected but the amount was too small to permit identification even when a litre of culture fluid was taken for the test.

Small amounts of a white, bulky precipitate, which resembled the "mucilaginous material" of Walker and Warren (14), were separated from the filtrate by alcohol and by acid precipitation. However, the material gave a negative test for uronic acids and hence was not identical with the precipitate that they obtained. The material was insoluble in hot water, dilute acid, and alkali but dissolved slowly in 60% sulphuric acid and was reprecipitated by diluting the solution with water, thus resembling cellulose.

Accordingly, a comparison was made of the rates of acid hydrolysis of the precipitated material and of very fine shreds of cellulose that had also been recovered from the fermentation. The precipitate was dried, and ground to a fine powder. It was then suspended in acetone to lyse adhering bacterial cells, and the acetone was removed. This was followed by extraction in the cold (3° C.) with 5% trichloroacetic acid and another washing with acetone, and the material was then dried *in vacuo*. The residual cellulose received a similar treatment with trichloroacetic acid.

The samples were hydrolyzed on a steam-bath with 2N hydrochloric acid, and the reducing sugar content of aliquot portions removed at intervals was determined according to Shaffer-Somogyi (12). The results given in Table I indicate more rapid hydrolysis with the precipitated material.

TABLE I  
RATES OF HYDROLYSIS OF ACID PRECIPITATED AND RESIDUAL CELLULOSE

Time, min.	Precipitate (Mgm. glucose per ml.)	Cellulose (Mgm. glucose per ml.)
15	0.44	Trace
30	0.54	0.28
60	0.56	—
120	0.63	0.45
180	0.73	0.45

Note: The purified material contained 7.2% nitrogen.

### *Pigment*

A greenish-yellow, fluorescing pigment resembling riboflavin was present in all cultures. This pigment could not be extracted with ethyl ether, petrol ether, chloroform, or ethyl acetate, but was extracted by benzyl alcohol in the presence of barium chloride (3), and by butyl alcohol saturated with sodium sulphate (8). It was adsorbed on freshly prepared lead sulphide and eluted with a mixture of 70 parts water, 2 parts glacial acetic acid, and 30 parts pyridine (8)—solvents used for extraction of riboflavin. These extracts showed the characteristic fluorescence of riboflavin, the presence of which was also demonstrated by a microbiological assay on the culture liquor (11).

The intensity of the fluorescence was diminished rapidly by ultraviolet radiation, by reduction with sodium hyposulphite, and to a lesser degree by oxidation with permanganate. The light absorption curve of the pigment in butyl alcohol showed a sharp rise in the range 4300 to 4000Å.

### *Carbon Dioxide*

An apparatus was designed for the gravimetric determination of the carbon dioxide produced in the cellulose decomposition. The carbon dioxide in the air entering the culture was removed by passage through a 50% solution of sodium hydroxide and then through soda lime and the carbon dioxide evolved in fermentation was absorbed on ascarite. All newly inoculated cultures failed to grow in this apparatus and it was believed that this could only be due to the removal of carbon dioxide from the incoming air. It was, therefore, decided to investigate the carbon dioxide requirements of the organisms.

### *II. Role of Carbon Dioxide*

An experiment showing that carbon dioxide is essential for the decomposition of cellulose by the organisms has already been described (10). Four culture flasks containing cellulose broth received a very small inoculum; two were aerated with normal air and two with carbon dioxide-free air. Inhibition of growth in the absence of carbon dioxide was also produced in the same manner with glucose and cellobiose as substrates. The addition of 1% calcium carbonate to glucose permitted growth in the flasks aerated with carbon dioxide-free air, but it was unsatisfactory for growth on cellulose. It has, therefore, not been possible to procure the necessary data for a carbon-balance sheet of cellulose decomposition.

The effect of concentrations of carbon dioxide above that of normal air was investigated using glucose broth as the substrate. An aspirator of the common type, with a capacity of 48 litres, was employed, and dilute acid was used as the displacing fluid. A blank determination was always included, the rates of bubbling being kept relatively constant for all tubes by means of precision pinchcocks. Growth was measured nephelometrically with an Evelyn colorimeter employing the 520 filter. A small inoculum was always used, the age of which was approximately the same for each trial.

The concentrations of carbon dioxide were varied between 1.2 and 10%. It was found that concentrations of 2.5 to 10% retarded growth but at 1.2% carbon dioxide, the growth was equal to that in air.

### III. The Influence of the Physical Nature of the Cellulose

The modern concept of the structure of cellulose holds that the closely-packed nature of the crystalline portion of cellulose renders difficult the penetration of chemical and physical agents between the micelles. On the other hand, the amorphous portion is more readily affected by these same agents. The latter should, therefore, be more accessible to bacterial enzymes.

Accordingly, cellulose was treated to increase its content of the amorphous portion, and the rate of decomposition compared with untreated cellulose. The treatment was carried out according to Assaf *et al.* (2), with the exception that 13% sodium hydroxide was used, since it was found to be the minimum concentration of alkali that would satisfactorily swell the cellulose.

A set of 200 ml. Erlenmeyer flasks containing the alkali-treated cellulose in salt solution was inoculated and cultured together with a set containing untreated cellulose. The results are illustrated in Fig. 1. There was more rapid decomposition of the alkali-treated cellulose, and these cultures produced considerably more pigment than the cultures containing untreated cellulose.

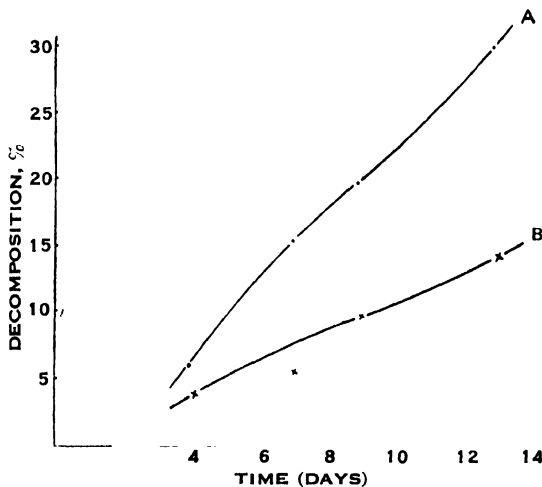


FIG. 1. Influence of the physical nature of the substrate on the decomposition of cellulose.  
A - Treated cellulose.  
B - Untreated cellulose.

### IV. Observations on Respiratory Enzymes

The Thunberg and Warburg techniques were employed at a temperature of 27° C. The first experiments were carried out with resting bacteria grown on starch or glucose broth. The Thunberg technique showed dehydrogenase activity with glucose and cellobiose, the former requiring slightly less time to decolorize the methylene blue. Cellulose prolonged the decoloration time.

Similar results were obtained by the 'direct' method of Warburg: the oxygen uptake with glucose as the substrate was slightly higher than with cellobiose, and cellulose gave negative results.

Cultures that were actively decomposing cellulose showed an oxygen uptake that was readily measurable with the respirometer. In this case samples of the cellulose with adhering bacteria were removed from the cultures with wide-mouth pipettes and used directly in the Warburg vessels, the hydrogen ion concentration of the cultures being first readjusted to about pH 6.5.

### *Effect of Carbon Dioxide*

The respiration of a 17 day old cellulose culture was measured by both the 'direct' and 'indirect' Warburg methods, that is, in the presence and in the absence of carbon dioxide. The results, illustrated in Fig. 2, show that the total oxygen uptake was greater in the presence of carbon dioxide.

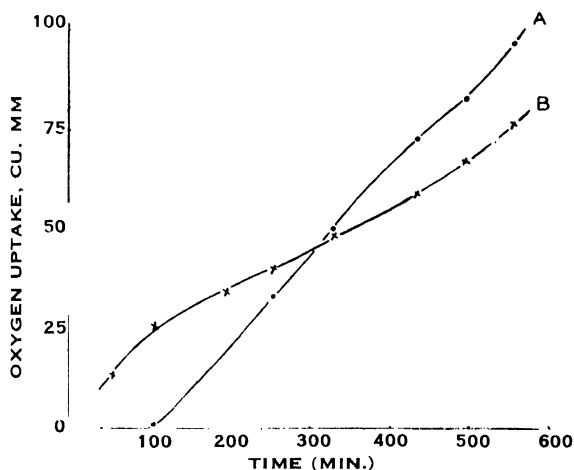


FIG. 2. Influence of the presence of carbon dioxide on the oxygen uptake of cellulose cultures.

A - Carbon dioxide present.

B - Carbon dioxide absent.

### *Effect of Adding Postulated Intermediates*

The effect of adding the postulated intermediates of cellulose decomposition—glucose and cellobiose—to cellulose was next investigated by the indirect method. The blank oxygen uptake of the cultures was determined for each vessel over a period of two to three hours so as to allow for differences in uniformity of the suspensions in different vessels. The substrates to be tested were then added from the side-arms, their final concentrations being about 1%. The results of one such experiment are given in Fig. 3. The bacteria-cellulose suspensions used in this experiment were from a 20 day culture. The stimulatory effect of glucose and the more marked stimulation by cellobiose were also observed by the direct method, and at 27° C. as well as at 37° C. Similar experiments with younger cultures, about eight days, did not show appreciable differences between the substrates added to cellulose.

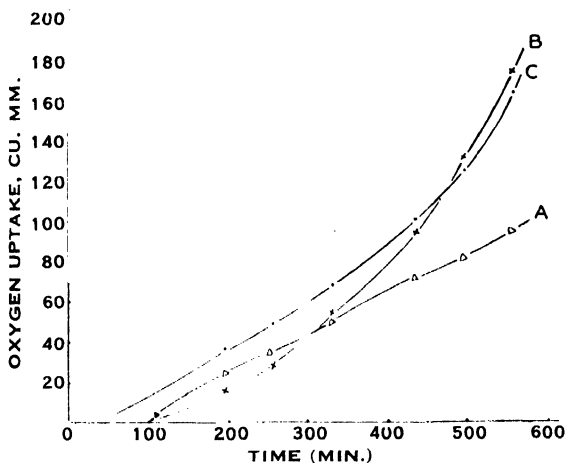


FIG. 3. Effect of the presence of glucose or cellobiose on the oxygen uptake of cellulose cultures.

A - Cellulose only.

B - Cellobiose added.

C - Glucose added.

#### V. Production of Reducing Sugars

A number of workers have demonstrated the formation of reducing sugars in cellulose cultures by employing various treatments designed to disrupt the normal activity of the organism (9). In some cases, however, these methods have failed to cause an accumulation of reducing sugars. The ability of the bacteria, used in this study, to produce reducing substances from cellulose was therefore examined.

Aliquots of cellulose cultures showing normal activity were adjusted to about pH 6.5, 5 ml. of toluene per 50 ml. of culture fluid were added, and the cultures were incubated at 26.6° C. Aliquots were removed periodically and reducing sugars determined by the Shaffer-Somogyi method.

The amount of reducing sugars varied considerably with the age and final hydrogen ion concentration of the cultures. In some cases the rate of formation was rapid and the final concentration of sugar was of a high order. For example, one culture showed an accumulation of 56.3 mgm. of reducing sugars per 50 ml. after four days' incubation.

##### (a). Identification of the Reducing Sugar

About 500 ml. of an active cellulose culture was incubated with toluene for five days, the cellulose filtered off and the culture liquid concentrated *in vacuo* to a small volume. To precipitate inorganic salt, 95% ethyl alcohol was added, the alcohol was distilled off, and the remaining liquid was clarified with charcoal. This solution yielded an osazone when heated for about 45 min. with phenylhydrazine hydrochloride and sodium acetate, the crystals having the characteristic appearance of glucosazone; only one type of crystal was present. Melting-point determinations were carried out with the unknown

osazone and glucosazone prepared from free glucose by the same method. Both gave melting points of 203° to 205° C., corrected (theoretical 204° to 205° C.). A mixed melting-point carried out with equal portions of the unknown and glucosazone gave a value of 205° C., corrected. The reducing sugar produced in the cellulose cultures was, therefore, glucose.

(b). *Effect of Dialysis on the Formation of Glucose*

The rate of glucose formation was generally found to decline gradually after a few days, and in some cases, dropped off sharply. The possibility was considered that accumulation of glucose might retard the additional formation of glucose by a mass-action effect. Dialyzing the sugar as rapidly as it is formed should, therefore, increase the total sugar produced over a given period.

Accordingly, aliquots of active cellulose cultures that were treated in the usual manner with toluene, were dialyzed in collodion sacs against a salt solution of equal salt concentration and the same hydrogen ion concentration; toluene was also added to the dialyzate.

Dialyzing 50 ml. aliquots of cellulose culture against 100 ml. and 200 ml. portions of salt solution did not show a significant increase in the total glucose produced in 4.5 days. The values obtained were 56.4 mgm. and 56.3 mgm., respectively, as compared with 53.6 mgm. produced by 50 ml. of the same culture incubated with toluene in an Erlenmeyer flask.

When dialysis was carried out in like manner against a larger volume of salt solution (400 ml. or 500 ml.) no reducing sugars were produced in the same period of time. In a further experiment, 50 ml. aliquots of a culture treated with toluene were dialyzed against 100 ml., 300 ml., and 500 ml. portions of salt solution, respectively. For comparison, another aliquot was incubated in an Erlenmeyer flask. The rates of glucose formation were determined with the results given in Table II.

TABLE II  
EFFECT OF DIALYSIS ON THE FORMATION OF GLUCOSE  
(Total mgm. glucose, including dialyzate)

Time, days	Untreated	Dialyzed against		
		100 ml.	300 ml.	500 ml.
1	11	0	0	0
2	20	21	Trace	0
4	29	27	33	0
5	—	43	40	—

These results suggested that some substance(s) produced by the bacteria, and necessary for glucose production was being removed by dialysis; and that, for glucose formation a sufficient concentration of the dialyzable factor

must be present to counteract the effect of dialysis. The hydrogen ion concentration inside the collodion membranes did not change appreciably and hence a Donnan equilibrium effect was considered unlikely.

Should a dialyzable factor be involved, then on discontinuing the dialysis the concentration should increase and glucose formation be resumed. To investigate this possibility, a culture that had been dialyzed against 500 ml. of salt solution and in which no glucose had been formed was divided into two portions. These were incubated with toluene and after 12 hr. reducing sugars were found to be present, the concentration being 0.2 mgm. per ml.

Further evidence that a dialyzable factor is involved in glucose formation was obtained as follows:

Two 50 ml. aliquots of a toluene-treated culture were dialyzed against 100 ml. and 300 ml. of salt solution, respectively. When the presence of glucose was detected in the 100 ml. dialyzate, equal portions of the culture that was being dialyzed against 300 ml. of salt solution and that contained no glucose were transferred to two test tubes. To Tube I was added 10 ml. of the 100 ml. dialyzate, which should have contained an appreciable concentration of the dialyzable factor, and to Tube II was added 10 ml. of a freshly-prepared salt solution. After eight hours' incubation, Tube I contained 3.5 mgm. glucose, whereas glucose was not present in Tube II. At 22 hr. the concentration in Tube I had increased to 4.6 mgm. and only a trace appeared in Tube II.

#### *VI. Optimum Hydrogen Ion Concentration for Activity with Cellulose, Glucose, and Cellobiose as Substrates*

If glucose and cellobiose are intermediates in the decomposition of cellulose, then it would be expected that the optimum pH would be the same for cellulose decomposition and for growth on glucose and cellobiose. Accordingly, the optimum pH for activity on these three substrates was determined.

The hydrogen ion concentration of the glucose and cellobiose broths was adjusted with *M*/15 phosphate buffer; the usual amounts of the other salts were used. On cellulose, growth was not obtained with *M*/15 buffer, hence it was necessary to use the normal, lower concentration of phosphate. Equal amounts of inoculum were used and the cultures were prepared in duplicate. With the sugars, growth was measured nephelometrically with an Evelyn colorimeter, using the 520 filter, and with cellulose the percentage decomposition was measured. Results of the experiment are illustrated in Fig. 4.

#### *VII. Inhibitors*

Four compounds commonly used as inhibitors in the study of carbohydrate metabolism were tested for their effect on activity of the organisms, i.e., fluoride, iodoacetate, azide, and phlorhizin.

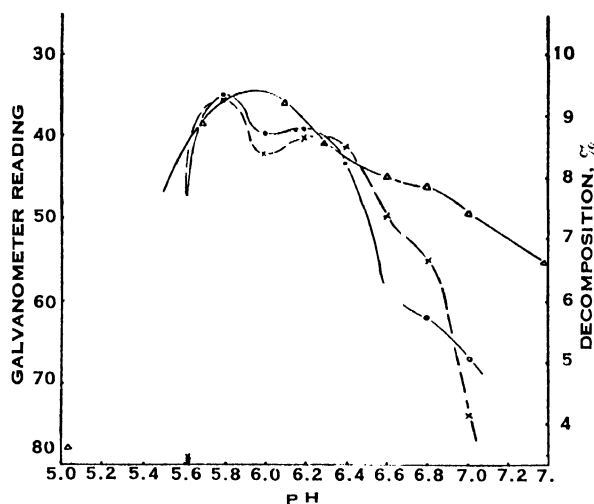


FIG. 4. Optimum hydrogen ion concentration for activity with cellulose, glucose, and cellobiose as substrates.

- $\Delta$  - Cellulose.  
 $\times$  - Cellobiose.  
 $\circ$  - Glucose.

(a). *Effect on Respiration of Cellulose Cultures*

This was studied with the Warburg apparatus (direct method). No effect was apparent until the bacteria-cellulose suspensions had been incubated with the inhibitors for several hours. Results of an experiment in which oxygen uptake was measured after 12 hr. incubation are given in Table III. The concentration of inhibitor was 1% and the temperature was 27° C.

TABLE III  
INHIBITION OF RESPIRATION OF A CELLULOSE CULTURE  
(Oxygen uptake in cu. mm.; Temp. 27° C.)

Time, min.	Untreated	Fluoride	Iodoacetate	Azide	Phlorhizin
15	7.5	2.8	3.9	3.4	2.2
75	27.7	5.6	5.9	7.4	2.7
105	37.8	5.6	6.5	8.1	4.4
165	55.4	6.7	6.5	12.8	5.0

The respiration of the cellulose culture was inhibited in large measure in all cases.

(b). *Effect on Growth*

Cellulose, glucose, and cellobiose broths were used as the substrates. The concentration of the inhibitors was 0.5% for cellulose and 0.25% for the latter two. The temperature was 26.6° C.



In each case, with the exception of fluoride, complete inhibition resulted; in the presence of fluoride there was a slight growth in glucose and cellobiose broth. The untreated cultures all showed normal growth.

(c). *Effect on the Production of Glucose*

Azide and phlorhizin could not be used in these experiments because they interfere with the determination of sugars. The other two compounds were added in concentrations of 1% to aliquots of cultures treated with toluene in the usual manner, and reducing sugars determined periodically. Results are given in Table IV. Iodoacetate completely inhibited the formation of glucose, while fluoride was partially inhibitory.

TABLE IV  
INHIBITION OF GLUCOSE FORMATION  
(Mgm. glucose per 100 ml.)

Time, hr.	Untreated	Fluoride	Iodoacetate
13	12	Trace	0
19	25	12	0
38	43	18	0

### Discussion

Aerobic, mesophilic bacteria have been characterized as yielding almost no products from cellulose other than carbon dioxide and synthesized cell materials (9). This also appears to be the case with the impure culture of *V. perimastix*. It has not been possible to determine the amount of carbon dioxide produced from cellulose by the bacteria, but it is obviously of a very high order since no other products appear in quantities large enough to explain the loss of cellulose.

The different rates of hydrolysis of the alcohol-acid precipitate and residual cellulose make it unlikely that the former is composed of short cellulose chains, but is possibly a synthesized bacterial polysaccharide. However, on hydrolysis the initial production of reducing substances from the precipitated material was rapid and then declined sharply, indicating that the material was not pure.

There is a striking similarity between the chemical and physical properties of the fluorescent pigment and riboflavin, so that the former is conceivably part of a flavin respiratory enzyme. If this is correct, the pigment would be expected to be present in large amounts in cultures showing rapid decomposition. The highest concentration of the pigment is obtained with alkali-treated cellulose, when the rate of decomposition is also most rapid.

Carbon dioxide has been found to be an essential growth factor for the bacteria and it is suggested that it plays some fundamental role in metabolic processes. Heterotrophic assimilation of carbon dioxide has been recognized

as an important process in the metabolism of several bacteria (15, pp. 135-182), and the list of organisms that require carbon dioxide continues to grow. This, however, appears to be the first instance to be reported of the essentiality of carbon dioxide in the case of aerobic cellulose-decomposing bacteria.

It is not known whether carbon dioxide is required for the breakdown of cellulose itself, or for the utilization of intermediate products. The carboxylation of pyruvic acid is the only known reaction that involves carbon dioxide assimilation (7), but this does not preclude other possibilities. Considering this reaction in the case of cellulose decomposition, the metabolic pathway of the polysaccharide would then, in part, coincide with that involved in the metabolism of many other carbohydrates.

The retarded growth observed in the presence of concentrations of carbon dioxide above that normally present in air may be due to the bacteria being adapted to lower carbon dioxide concentrations. In penicillin fermentations it has been found that varying the concentration of carbon dioxide affects the activity when the gas mixtures are bubbled through the cultures, but not when the cultures are merely shaken in an atmosphere containing the same gas mixtures (6). No explanation is given, but this observation indicates that the results may depend on the type of treatment.

There is a marked increase in the oxygen uptake with cellulose in the presence of carbon dioxide over that in its absence, which emphasizes the importance of carbon dioxide in the metabolism of the bacteria. The greater initial respiration rate in the absence of carbon dioxide is not explained.

The demonstration of the presence of appropriate dehydrogenases and the more rapid oxygen uptake with glucose would be in accord with the postulated role of glucose and cellobiose, respectively, as intermediates. Furthermore, the pH activity curves on glucose, cellobiose, and cellulose are somewhat similar, and glucose is also produced in fairly large amounts when the decomposition takes place in the presence of toluene. The increase in the respiration of an active cellulose-decomposing culture when cellobiose or glucose are added may also be significant. If the extra oxygen uptake is merely due to decomposition of the added glucose or cellobiose, then the respiration would be expected to follow a course similar to that with glucose or cellobiose alone. This is not the case, however, for the oxygen uptake in the absence of cellulose is greater with glucose than with cellobiose, and in the presence of cellulose the results are reversed.

These observations do not necessarily prove that glucose or cellobiose are normal intermediates in the decomposition of cellulose. Nevertheless, they strongly suggest a similarity in the metabolism of the bacteria on the three substrates. It is possible that phosphorylation mechanisms are involved, since phosphorylation inhibitors prevent growth on these carbohydrates, inhibit respiration of cellulose cultures, and retard the production of reducing sugars. In such a case, glucose, cellobiose, and cellulose could have common

pathways of dissimilation, and glucose and cellobiose could accumulate if the normal decomposition is disturbed, since they would be in equilibrium with other intermediates of cellulose decomposition. Stanier (13) has suggested that phosphorolytic decomposition is a possibility.

Glucose is produced in relatively large amounts when an active cellulose culture is treated with toluene. It is likely that a dialyzable factor, possibly a coenzyme produced by the bacteria, is involved in the production of glucose under these conditions. The formation of reducing sugars when toluene is present has been demonstrated with a number of other bacteria, but it has not previously been indicated that a dialyzable factor is involved. When the nature of this factor is known it may be possible to elucidate the mechanism of glucose formation, and possibly obtain a clearer insight into the nature of the enzymic processes involved in cellulose decomposition.

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# A FORM OF *LEPTOSPHERA AVERNARIA* ON WHEAT IN CANADA<sup>1</sup>

By T. JOHNSON<sup>2</sup>

## Abstract

In the late summer of 1942, a *Septoria* sp. with spores longer than those of *Septoria nodorum* Berk., and conforming rather closely to those of *S. Avenae* Frank, was found commonly in the Prairie Provinces of Canada on leaves of wheat and, in one instance, on leaves of barley. It was found again on wheat and more rarely on barley in both Eastern and Western Canada each year from 1943 to 1946. Occasionally, perithecia, attributable to the genus *Leptosphaeria*, were found on leaves that previously had borne numerous pycnidia of this organism. That the perithecia represented the perfect stage of this *Septoria* sp. was shown by the development of similar perithecia in many cultures established from pycnidiospores, and by the formation of pycnidia in certain cultures grown from ascospores of perithecia collected on wheat. The perithecia, asci, and ascospores conform closely though not identically with the description of *Leptosphaeria avenaria* Weber.

The organism under discussion differs from *S. nodorum* not only in the size of its pycnidiospores but also in its inability to cause glume blotch, its longer incubation period, and lack of ability to attack the seedlings of certain wheat varieties susceptible to *S. nodorum*. It differs from *S. Avenae* in symptoms, host range, length of incubation period, and cultural characteristics. It differs from both these species by a lower parasitic vigour and a marked tendency to develop on fading leaves and sheaths. Owing to its morphological similarity to *L. avenaria* it is here described as a *forma specialis* of that species.

## Introduction

During the late summer and fall of 1942, pycnidia of *Septoria* spp. were more than usually common on wheat leaves in the Prairie Provinces of Western Canada. In most of the samples examined by the writer, at least of those collected in Manitoba and Saskatchewan, the pycnidiospores did not conform to those of either *Septoria nodorum* Berk. or *S. Tritici* Desm. Pycnidia, though abundant on the leaves in many localities, occurred only rarely on the heads. The purplish-brown blotching of glumes and lemmas characteristic of wheat heads infected by *S. nodorum* was rarely seen although the extreme margins of the glumes were frequently brown and the terminal parts of glumes and lemmas often showed a faint, light brown discoloration. In some specimens of wheat examined, the pycnidiospores conformed closely to the description given for *S. nodorum* (1, 2, 5, 12) and were undoubtedly attributable to that species. In most specimens, however, the maximum length of the spores greatly exceeded the dimensions given for *S. nodorum*, the spores in many specimens reaching a maximum length of between 45  $\mu$  and 50  $\mu$  whereas the maximum length given for *S. nodorum* by any of the authors cited above is 36  $\mu$ . The spores were cylindrical, straight or slightly sinuate, rounded

<sup>1</sup> Manuscript received July 29, 1947.

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<sup>2</sup> Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

at the ends, three-septate or rarely four-septate, and in general, bore a resemblance in size and shape to the pycnidiospores of *Leptosphaeria avenaria* Weber (*Septoria Avenae* Frank) (see Figs. 2 and 3).

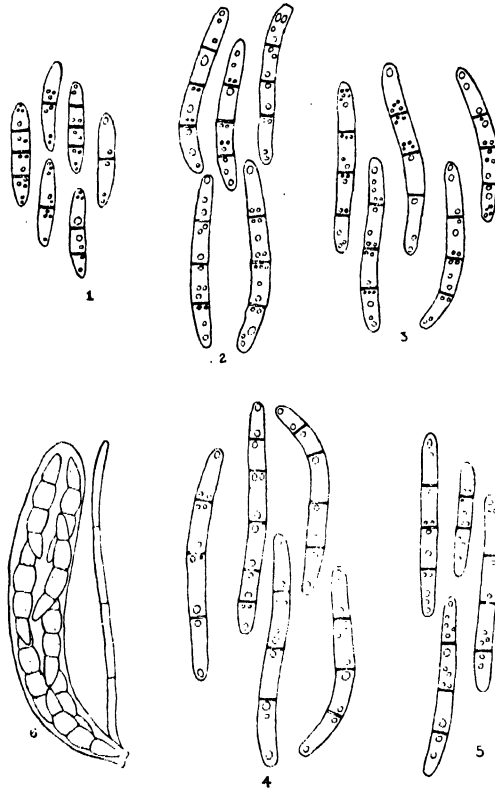


FIG. 1. *Septoria nodorum*—pycnidiospores collected on wheat leaves at Kentville, N.S., 1946.

FIG. 2. *Leptosphaeria avenaria* f. sp. *triticea*—pycnidiospores collected on wheat leaves, Carlyle, Sask., 1946.

FIG. 3. *Leptosphaeria avenaria* f. sp. *triticea*—pycnidiospores collected on barley leaves, Morden, Man., 1945.

FIG. 4. *Septoria Avenae*—pycnidiospores collected on oats, Normandin, Que., 1946.

FIG. 5. *Septoria Avenae*—pycnidiospores collected on oats, Fort William, Ont., 1946.

FIG. 6. *Leptosphaeria avenaria* f. sp. *triticea*—ascus from a perithecium that developed in a culture originating from pycnidiospores that, in turn, developed in a culture grown from a perithecium collected on wheat at Wilcox, Sask., 1942.

Magnification: Figs. 1 to 5, about 680  $\times$ ; Fig. 6, about 590  $\times$ .

The same organism was again found, in 1943, on wheat specimens from both Western and Eastern Canada, but only in trace quantities. In 1944 it was widespread on wheat in Manitoba, but infection was mostly light though heavy infection was noted in several localities in the latter part of August. In that

year it was also found on wheat specimens in Ontario, Quebec, and the Maritime Provinces. In 1945 it caused widespread infection on wheat in Manitoba and the abundance of infection on fading leaves suggested that the death of the leaves was hastened by the infection. Slight to moderate infections were also noted in several localities in Eastern Canada. Infection of wheat in Manitoba was general but light in 1946. In that year, it occurred also at a number of places in the other two Prairie Provinces as well as in many localities in Eastern Canada.

### Occurrence of the Perfect Stage

When pycnidia of this *Septoria* sp. were first observed in 1942, perithecia attributable to *Leptosphaeria* were occasionally found associated with them. This same association of pycnidia and perithecia was noted in several instances in 1943 and 1944. In the latter year, abundant perithecial development was observed at Winnipeg and at Gimli, Man. on wheat leaves bearing numerous pycnidia. At Winnipeg, perithecia were first seen on Aug. 25 on leaves of Gaza wheat. At that time they were still immature. On Sept. 7 the perithecia were very abundant and quite mature. Perithecia have also been found in 19 separate cultures derived from pycnidiospores, and pycnidia have been found in six different cultures derived from ascospores. The growth characteristics and pathogenicity of cultures originating from pycnidiospores and ascospores are so similar that it is clear beyond doubt that the perithecia represent the perfect stage of the *Septoria* sp.

### Nomenclature

As information concerning this organism accumulated, the question of its nomenclature naturally arose. It was soon decided that it was readily distinguishable on the basis of the morphology of the pycnidiospores from *S. Tritici* and *S. Passerinii* Sacc. and from *S. graminum* either as originally described by Desmazieres\* or as amended by Sprague (7); and is probably also distinguishable without difficulty from *S. Secalis* Prill. & Del., which, according to Weber (13), has somewhat narrower, curved pycnidiospores. It is separable from *S. nodorum* by the size and, to a certain extent, the shape of the pycnidiospores, and is also pathogenically different.

Of the wheat-inhabiting *Septoria* species the one that appears to resemble most closely the organism under discussion is *S. trititicola* Lobik, collected in the vicinity of Piatigorsk, U.S.S.R. (3). The symptoms and the dimensions of the pycnidia and spores agree approximately but the spores of the Russian species are described as three- to five-septate, slightly curved, and gradually tapering from base to tip, whereas the Canadian organism is generally three-septate with mostly straight spores that show little or no tapering. Furthermore, *S. trititicola* has no known perfect stage and so long as that is unknown it seems undesirable to identify the Canadian organism with it.

\* See Saccardo, P. A. *Sylloge fungorum. III. Patavii. 1884.*

The organism here discussed is probably not morphologically distinguishable in either its perfect or imperfect stage from *Leptosphaeria avenaria* but is strikingly different pathogenically. The pycnidiospores appear also to be similar to those of *Stagonospora arenaria* Sacc., which according to Sprague (8), bear a close resemblance to those of *Septoria Avenae*. Although no cultures of *Stagonospora arenaria* were available for pathogenicity tests, that species is probably pathogenically distinct from the organism under discussion, which showed no ability, in green-house tests, to attack *Arrhenatherum elatius*, *Dactylis glomerata*, *Elymus canadensis*, or *Phalaris arundinacea*, which are reported to be hosts of *S. arenaria* (8).

The above considerations raise the question of where this *Septoria* sp. with its connected *Leptosphaeria* is to be placed taxonomically. There appear to be two alternatives. One is to describe it as a new species. Perhaps the chief objection to this course is that, if it were so described, it would be the only species of *Septoria* on cereals that could not be rather readily distinguished on a purely morphological basis. The other is to regard it as a *forma specialis* of the species it most resembles morphologically, namely, *Leptosphaeria avenaria*. As morphology rather than physiology is the basis of specific determination, the latter course seems the safest to follow. A somewhat similar course has been followed by Sprague who described (9) as *S. Tritici* Rob. f. *Avenae* (Desm.) Sprague, a narrow-spored type of *Septoria* on oats that he had originally (6) considered a physiologic form of *S. Tritici*. It is therefore suggested that the wheat-inhabiting *Septoria* here under discussion be regarded as a *forma specialis* of *Leptosphaeria avenaria*.

## Description

*Leptosphaeria avenaria* WEBER, F. SP. *triticea* F. SP. NOV.

Maculis ovatis, saepe confluentibus, pallide luteolis, in media parte griseis. Peritheciis initio epidermide tectis dein erumpentibus, atris, globosis vel subglobosis, 100-220  $\mu$  dia., ostiolo circulari, 14-20  $\mu$  dia.; ascis clavatis, rectis vel paulum curvatis, hyalinis, 40-80  $\times$  8-11  $\mu$ , octosporis, ascosporis biseriatis, fusoides, rectis vel curvatis, 3-septatis, saepe ad septa constrictis, pallide luteolis (16) 19-25 (28)  $\times$  4-6  $\mu$ ; paraphysibus filiformibus, septatis, paulo longioribus quam ascis.

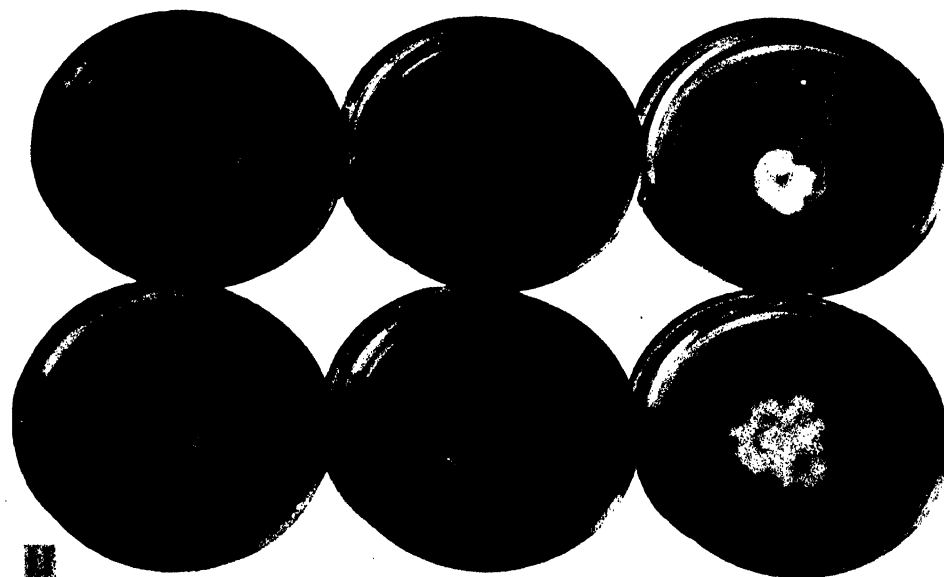
FIG. 7. Asci and spores of *Leptosphaeria avenaria* f. sp. *triticea* collected on Apex wheat leaves, Wilcox, Sask., 1942. About 495  $\times$ .

FIG. 8. Ascospores of *L. avenaria* f. sp. *triticea* formed on cornmeal agar. About 495  $\times$ .

FIG. 9. Young lesions of *L. avenaria* f. sp. *triticea* on Pelissier wheat, Winnipeg, Man., 1945.

FIG. 10. Cross section of pycnidium of *L. avenaria* f. sp. *triticea* collected on Gaza wheat, Aug. 18, 1942. About 295  $\times$ .

FIG. 11. Twelve-day old colonies of *S. nodorum* (left), *L. avenaria* f. sp. *triticea* (centre), and *S. Avenae* (right) on potato dextrose agar.







STAT. PYCNID. *Septoria Avenae* FRANK F. SP. *triticea* F. SP. NOV.

Pycnidiis initio epidermide tectis dein erumpentibus, globosis vel subglobosis, atris (80) 90-140 (210)  $\mu$  dia. cellis superficialibus polygoniis; ostiolo circulari vel ovato, 10-15  $\mu$  dia.; sporulis chlorino-hyalinis, cylindratis, rectis vel leniter sinuosis, utrinque obtusiusculis, 3-septatis vel raro 4-septatis (18) 26-42 (53)  $\times$  (2.3) 2.8-3.5 (4.2)  $\mu$ . Sporulis paulo brevioribus et angustioribus quam in *Septoria Avenae*.

Hab. in foliis vaginisque *Tritici vulgaris*, *Tritici duri* et rarius in foliis *Hordei vulgaris*, in Canada.

*Leptosphaeria avenaria* WEBER F. SP. *triticea* F. SP. NOV.

Lesions on leaves ovate, frequently coalescing, straw color or buff, central portion bearing pycnidia often gray. Pycnidia sometimes followed in late summer by perithecia.

Perithecia at first subepidermal, later erumpent, black by reflected light, brown by transmitted light, globose or subglobose, 100-220  $\mu$  in diameter, surface cells polyhedral, often pentagonal; ostiole circular 14-20  $\mu$  in diameter; asci clavate, straight or slightly curved, hyaline, 40-80  $\times$  8-11  $\mu$ , eight-spored, ascospores biserially arranged, fusoid, straight or curved, three-septate, frequently constricted at the septa, light yellow (16) 19-25 (28)  $\times$  4-6  $\mu$ ; paraphyses filamentous, septate, slightly longer than the asci, hyaline.

On leaves and sheaths of wheat (*Triticum vulgare*, *Triticum durum*).

Type—Winnipeg, Man. Sept. 7, 1944. DAOM 19545.

PYCNIDIAL STAGE *Septoria Avenae* FRANK F. SP. *triticea* F. SP. NOV.

Pycnidia at first subepidermal, later erumpent, scattered, spherical or slightly elongate, black by reflected light, golden brown by transmitted light (80) 90-140 (210)  $\mu$  in diameter, surface cells polyhedral; wall composed of three or four layers of brown cells, 7-11  $\mu$  thick at base and 11-14  $\mu$  thick at top of pycnidium; ostiole circular or oval, 10-15  $\mu$  in diameter; pycnidiospores hyaline to faintly chlorinous, thin-walled, cylindrical, straight or slightly sinuate, obtuse or rounded at the ends, three-septate or rarely four-septate, (18) 26-42 (53)  $\times$  (2.3) 2.8-3.5 (4.2)  $\mu$ . Spores generally somewhat shorter and more slender than in *Septoria Avenae*.

On leaves and sheaths of wheat (*Triticum vulgare*, *Triticum durum*) and, more rarely, on leaves of barley (*Hordeum vulgare*).

Type—Winnipeg, Man. Aug. 4, and Aug. 25, 1944 on *Triticum durum*, variety Gaza. DAOM 19546.

### Host Range

Although *L. avenaria* f. sp. *triticea* is primarily a wheat-inhabiting organism, it is nevertheless found frequently on other cereals, particularly barley. Its presence was first noted on barley in 1942 when a bundle of heavily infected wheat from Indian Head, Sask. was found to contain two culms of barley, the

leaves of which bore numerous pycnidia. In 1943, pycnidia were found on barley leaves in 10 separate localities distributed in the provinces of Alberta, Saskatchewan, Manitoba, Ontario, Quebec, and Prince Edward Island. In no case, however, was the infection more than a mere trace. In 1944, the same organism was found on barley leaves at Winnipeg, Man., and in two localities in each of the provinces of Ontario, Quebec, and Nova Scotia. Infections were a mere trace except at Winnipeg where heavy pycnidial development occurred in August on the leaves of many barley varieties in the experimental plots. This infection, however, was somewhat lighter than in adjacent wheat plots. Barley was also found infected in 1945 and 1946 but never in more than trace amounts.

Less frequently than on barley, pycnidia of f. sp. *triticea* have been found on leaves and sheaths of oats, rye, and certain grasses, particularly species of *Elymus* and *Agropyron*, but as the pycnidia occurred chiefly on senescent plant parts it is doubtful whether these are congenial hosts. Examination and measurement of pycnidia and spores have shown the organism collected on these hosts to be morphologically the same as that collected on wheat and barley, and pathogenic tests have invariably shown that the cultures collected on oats and grasses as well as on barley attack wheat more readily than the host from which they were obtained. In fact, so rarely have infection tests with f. sp. *triticea* produced any symptoms whatsoever on barley, oats, or grasses that it seems probable that these hosts are highly resistant except when in a nearly mature or senescent condition.

### Comparisons of *L. avenaria* f. sp. *triticea* with *Septoria nodorum* and *Septoria Avenae*

As soon as studies on *L. avenaria* f. sp. *triticea* were undertaken attempts were made to compare it morphologically and pathogenically with *Septoria nodorum* and *S. Avenae*. A few of many such tests are summarized in Table I.

#### *Comparison of Morphological Characteristics*

It was soon realized that f. sp. *triticea* and *S. nodorum* could not be distinguished by visual inspection of infected wheat leaves. Many specimens collected in Eastern Canada had both organisms present on the same leaves and mass scrapings from such leaves consequently contained spores of both. Scrapings from infected heads, however, almost invariably contained only the typical short-spored *S. nodorum* with pycnidia 80 to 140  $\mu$  in diameter and slightly curved, one- to three-septate, hyaline spores 15 to 25  $\times$  2.5 to 3.5  $\mu$ . When pycnidia were detached singly from the leaves it was not possible to determine their specific identity until their spore contents were examined. It then became readily apparent that any given pycnidium contained either spores characteristic of *S. nodorum*, as described above, or spores of a somewhat different shape and considerably larger size. These spores were usually straight or slightly sinuous, cylindrical though often thickest at the base, three-septate, about 25-40  $\times$  2.5-3.5  $\mu$ . Both as to dimensions and shape these spores were readily distinguishable from those of *S. nodorum*.

Evidence from comparisons of the pycnidial stage of *L. avenaria* f. sp. *triticea* with *S. Avenae* indicates that these two species cannot be distinguished readily on the basis of morphology. Over 200 measurements of pycnidia of f. sp. *triticea* collected on wheat and barley and about 60 measurements of pycnidia of pathogenically tested collections of *S. Avenae* gave the following dimensions (the extreme limits of size are shown in parentheses).

*F. sp. triticea* (80) 90-140 (210)  $\mu$ . Ostioles mostly 10-15  $\mu$ .

*S. Avenae* (83) 110-150 (230)  $\mu$ . Ostioles about 15  $\mu$ .

Pycnidia of both organisms were fuscous to black when seen with a lens but brownish yellow to light brown when viewed with a microscope.

Measurements of spores from 47 samples of f. sp. *triticea* collected on wheat, and 24 samples of *S. Avenae* gave the dimensions below:

f. sp. *triticea* (18) 26-42 (53)  $\times$  (2.3) 2.8-3.5 (4.2)  $\mu$ .

*S. Avenae* (21) 29-45 (61)  $\times$  (2.5) 2.9-3.9 (4.5)  $\mu$ .

These data indicate that both pycnidia and spores of *S. Avenae* are slightly larger than those of f. sp. *triticea* but in view of the similarity in appearance of the pycnidia and of the spores of the two organisms, the size differences are insufficient to permit of ready distinction between them.

Comparison of the perfect stage of f. sp. *triticea* with that of *S. Avenae* is rendered difficult by the fact that the perithecia of *S. Avenae* (*Leptosphaeria avenaria*) have not been found on field material and are known only from Weber's description (11) of perithecia and ascospores formed in cultures growing on agar. The following is a comparison of the dimensions of perithecia, asci, and ascospores of *L. avenaria* f. sp. *triticea* with corresponding dimensions of *L. avenaria* given by Weber.

#### *L. avenaria* f. sp. *triticea*

Perithecia (in nature) 110-150 (220)  $\mu$ . Ostiole 14-20  $\mu$ .

Perithecia (in culture) about 140 (220)  $\mu$ .

Asci (in nature) (42) 50-70 (78)  $\times$  (7) 8-10 (11)  $\mu$ .

Asci (in culture) (36) 50-70 (85)  $\times$  (6) 8-11  $\mu$ .

Ascospores (in nature) (16) 19-25 (27)  $\times$  (3.9) 4.5-5.5 (6.0)  $\mu$ .

Ascospores (in culture) (16) 19-25 (28)  $\times$  (3.0) 4.0-5.0 (5.3)  $\mu$ .

#### *L. avenaria* (according to Weber)

Perithecia (in culture) 60-130  $\mu$ . Ostiole 12-20  $\mu$ .

Asci (in culture) 30-100  $\times$  10-18  $\mu$ .

Ascospores (in culture) 23-28  $\times$  4.5-6.0  $\mu$ .

Weber's data indicate for *L. avenaria* somewhat smaller perithecia and wider asci and ascospores than in *L. avenaria* f. sp. *triticea* but it is doubtful whether much significance should be attached to these differences.

### *Comparison of Pathogenic Characteristics*

In host range and symptoms *f. sp. triticea* and *S. Avenae* are quite distinct. The latter, which is actively parasitic on oats, produces rather large chocolate-brown or purplish-brown, oval lesions in which pycnidia are present though usually not readily visible against the pigmented lesion.

*F. sp. triticea* has been found to occur on oats but in all the known instances it was present on plant parts (leaves or upper parts of sheaths) that had reached maturity and was not accompanied by the characteristic brown lesions of *S. Avenae*. It is evidently not actively parasitic on oats although it is possible that it can become established on fading plant parts.

Comparative tests were made of the pathogenicity of *f. sp. triticea*, *S. nodorum*, and *S. Avenae* by infecting seedling plants of wheat, oats, barley, and rye. In these tests two different methods were used: (1) Infection with spore suspensions obtained directly from infected plants gathered in the field. (2) Infection with pure cultures of the organisms, each culture commonly established from the spores of a single pycnidium.

The two methods produced similar results but the first (the use of spores gathered directly from field material) had the disadvantage that other parasitic organisms were sometimes present on the field material from which the spore suspensions were secured. The majority of the infection tests were therefore carried out with pure cultures of monopycnidial origin, the inoculum being produced on finely ground oat hulls. The ground oat hulls were placed in test tubes, moistened with water, autoclaved, and inoculated with spores or mycelium. Later, when the mycelial growth had penetrated to some depth in the tubes, part of the contents was removed, macerated in water, and applied to the leaves with the fingers. Following inoculation, the seedling plants were kept in moist chambers for a period of two or three days. By this method it was found that the pathogenicity of even non-sporulating cultures could be tested readily.

*F. sp. triticea* proved pathogenically much weaker on wheat than did *S. nodorum*, which normally destroyed inoculated leaves in six or seven days. In that time, *f. sp. triticea* produced scarcely any visible symptoms. Four or five days later, however, numerous necrotic areas usually developed. These generally took the form of small oval lesions of buff color, sometimes with a darker centre. A large number of infection experiments has indicated that the incubation period of *f. sp. triticea* is about twice as long as that of *S. nodorum*, or approximately 10 days while that of *S. nodorum* is five days.

*F. sp. triticea* differs furthermore from *S. nodorum* by its inability to attack, in the seedling stage, certain wheats highly susceptible to the latter. The virtual immunity of seedling leaves of the varieties McMurachy and Regent from attack by *f. sp. triticea* proved a useful means of distinguishing that species from *S. nodorum*, which attacks these varieties vigorously.

Neither f. sp. *triticea* nor *S. nodorum* was able to attack seedlings of barley, rye, or oats. The inability to attack oats distinguished these species sharply from *S. Avenae*, which was extremely pathogenic to oat seedlings on which it produced typical, oval, brown lesions in four or five days after inoculations (Table I).

TABLE I

PYCNIDIOSPORE DIMENSIONS AND PATHOGENICITY ON CEREAL SEEDLINGS OF *Leptosphaeria avenaria* F. SP. *triticea*, *L. avenaria*, AND *Septoria nodorum*

Culture No.	Source		Spore size, $\mu$		Pathogenicity (% of terminal three inches of seedling leaf killed)					
			Range	Mean length	Wheat			Oats	Barley	Rye
					Gaza	Marquis	Regent			
<i>L. avenaria</i> f. sp. <i>triticea</i> —from pycnidia on wheat										
2	Winnipeg, Man.	1942	28-42	$\times$ 3.0-3.6	34	8	6	0	0	0
103*	"	1943	28-49	$\times$ 2.6-3.3	37	7	4	3	0	0
142	"	1944	27-42	$\times$ 2.8-3.6	34	19	5	0	0	0
143*	Morden, Man.	1944	29-43	$\times$ 3.1-3.6	36	6	5	1	0	0
150	Guelph, Ont.	1944	22-44	$\times$ 2.8-3.6	30	33	14	6	0	0
233	Indian Head, Sask.	1945	22-41	$\times$ 2.8-3.6	34	2	1	0	0	0
225	Kemptville, Ont.	1945	20-37	$\times$ 3.1-3.3	30	5	6	0	0	0
385	Kapuskasing, Ont.	1945	39-50	$\times$ 2.8-3.3	44	12	5	0	0	0
<i>L. avenaria</i> f. sp. <i>triticea</i> —from perithecia on wheat										
33*	Wilcox, Sask.	1942	Ascospores 20-25	$\times$ 4.4-5.6	2	1	0	0	0	—
46*	"	1942			5	8	0	0	0	—
157	Winnipeg, Man.	1944			54	17	6	0	0	0
158*	"	1944			8	5	1	0	1	0
161	"	1944	Ascospores 22-25	$\times$ 5.6	16	10	0	0	0	0
173	Gimli, Man.	1944			16	3	0	0	0	0
<i>L. avenaria</i> f. sp. <i>triticea</i> —from pycnidia on barley										
12	Indian Head, Sask.	1942	20-38	$\times$ 2.6-3.3	26	7	5	2	0	0
100	Winnipeg, Man.	1943	24-35	$\times$ 2.4-3.0	29	14	0	0	0	0
141*	"	1944	28-40	$\times$ 2.8-3.3	34	6	4	1	0	0
228	"	1945	30-42	$\times$ 2.8-3.9	37	3	1	0	0	0
320	St. Norbert, Man.	1946	28-40	$\times$ 2.8-3.3	35	tr.	0	0	0	0
<i>S. nodorum</i>										
77	Wilcox, Sask.	1942	17-24	$\times$ 3.0	21	34	55	60	0	0
113	Ottawa, Ont.	1943	17-27	$\times$ 2.4-3.7	23	46	41	36	0	1
151	Guelph, Ont.	1944	17-28	$\times$ 2.8-4.2	20	54	49	52	0	0
153	Lennoxville, Que.	1944	17-28	$\times$ 2.8-3.3	20	70	76	83	0	0
204	Winnipeg, Man.	1945	20-32	$\times$ 2.8-3.3	27	46	42	55	0	tr.
360	Fredericton, N.B.	1946	18-22	$\times$ 2.8-3.3	20	40	35	54	0	0
<i>L. avenaria</i>										
176	Fort William, Ont.	1944	24-40	$\times$ 3.1-4.5	34	0	0	0	45	0
238	Kapuskasing, Ont.	1945	28-39	$\times$ 2.8-3.6	33	0	0	0	15	0
213	St. Catharines, Ont.	1945	34-48	$\times$ 3.1-3.6	41	0	0	0	37	0
232	Guelph, Ont.	1945	27-35	$\times$ 2.8-4.2	30	0	0	0	6	0
362	Kentville, N.S.	1946	25-43	$\times$ 2.8-3.3	36	0	0	0	8	0
375	Normandin, Que.	1946	31-46	$\times$ 2.8-3.9	40	0	0	0	6	0
382	Kapuskasing, Ont.	1946	36-61	$\times$ 2.5-3.9	49	0	0	0	6	0

\* Perithecia formed in culture.

### Comparison of Cultural Characteristics

Tests on potato dextrose, cornmeal, and malt agar have shown that *f. sp. triticea* differs but little from *S. nodorum* in growth characteristics but may be distinguished from *S. Avenae* rather readily.

Both *f. sp. triticea* and *S. nodorum* produce at first a fine-textured, creamy-white, cottony mycelium that later assumes a buff color. The aerial mycelium of *f. sp. triticea* is usually rather coarser in texture, more tufted, and the substratum shows a more marked tendency to develop a dark-green or bluish-green pigment. Despite these apparent differences, cultures of the two organisms are not always readily distinguishable owing, largely, to the considerable variation between different cultures of the same species. Both organisms normally produce round colonies with uniformly smooth edges.

Colonies of *S. Avenae* are definitely slower in growth, and generally produce a cushiony aerial mycelium of a pure white color with little or no pigmentation in the substratum. The margins of the colonies are commonly ragged or irregular in appearance (Fig. 11).

### Discussion

There is no reason to suppose that the organism here described as *Leptosphaeria avenaria f. sp. triticea* has made its appearance only in the last few years. Examination of herbarium specimens has proved the presence on wheat in past years of the pycnidial stage of this species. One such specimen collected on wheat at Winnipeg in 1927 was provisionally identified as *Septoria Tritici*, presumably because the pycnidiospores were too long to fit the description of *S. nodorum*. Another similar specimen was collected on wheat at Rosthern, Sask., in 1928 by Dr. R. C. Russell and submitted to Dr. G. R. Bisby for identification. The latter labelled the specimen *S. nodorum* but noted that the dimensions of the spores were  $25.2-43.2 \times 3-4 \mu$ . Dr. R. Sprague also assigned to *S. nodorum* a large-spored type of *Septoria* probably similar to the one under discussion (10). The pycnidial stage of *L. avenaria f. sp. triticea* may also have been attributed by some collectors to *Stagonospora arenaria* Sacc. the pycnidiospores of which, according to Sprague (8), bear a close resemblance to those of *Septoria Avenae*, which in turn resemble those of *f. sp. triticea*.

The reason why *f. sp. triticea* has not been previously recorded as a separate entity lies perhaps in the inconspicuous nature of the symptoms, a certain resemblance of the pycnidia and spores to those of *S. nodorum*, and the fact that the two organisms sometimes occur together on the same plant.

The disease caused by *f. sp. triticea* is probably of minor economic importance. In 1942, 1944, and 1945 it caused considerable leaf necrosis on wheat in Manitoba but as this occurred rather late each season it is doubtful whether it led to much reduction in yield. The organism appears to develop best on leaves approaching the senescent stage, a fact that, taken in conjunction with the slow and, in comparison with *S. nodorum*, limited development of necrotic

areas in greenhouse tests, would indicate a relatively weak parasitism. Its long incubation period no doubt slows up its development in early and mid summer and probably prevents the appearance of any sudden epiphytotics.

That pathogenicity on barley is of a still lower order is shown by the rare development of symptoms on barley seedlings in greenhouse tests and by the absence in the field of heavy or even moderate attacks except in late summer on leaves already senescent.

*L. avenaria* f. sp. *triticea* does not ordinarily produce visible symptoms on the heads of either wheat or barley. There is, nevertheless, evidence that infection of the heads of both cereals does take place. During the course of the work here reported Dr. J. E. Machacek turned over to the writer many cultures of undetermined *Septoria* species isolated from seed of wheat, barley, and oats grown in various parts of Canada (4). These were found to fall into two classes (i) typical *S. nodorum*, collected most frequently in Eastern Canada, and (ii) f. sp. *triticea*, most frequently collected in Western Canada.

From Dr. Machacek's extensive isolation of *Septoria* cultures from wheat and barley seed collected in all the provinces of Canada over the period 1939-1943 it is evident that *S. nodorum* shows a strong tendency to infect wheat seed in preference to barley seed. In the Maritime Provinces, where that species is common, *Septoria* cultures (species not always determined) were isolated 26 times more frequently from wheat seed than from barley seed. In the Prairie Provinces where f. sp. *triticea* is common, *Septoria* cultures were isolated twice as often from barley seed as from wheat seed, which indicates that the hull of the barley seed is a favorable medium for the establishment of the fungus, perhaps saprophytically. The occurrence of f. sp. *triticea* on seed is probably a reflection of its tendency to invade mature tissues. Whether such invasion constitutes a means of overwintering for the organism is not certain. Greenhouse studies have shown that coleoptile lesions will occur on plants growing from infected seed but there is little or no proof of further spread to the growing plant.

### Acknowledgments

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## Phosphorus Deficiency in Relation to the Nitrate Reduction Test

In classifying bacteria, the ability to reduce nitrates to nitrites is an important characteristic. The *Manual of Methods for the Pure Culture Study of Bacteria* (1) recommends two media, one for organisms requiring organic nitrogen, the other for soil bacteria and related organisms not needing organic nitrogen.

Both media have been used in this laboratory in testing certain soil bacteria, long suspected of being capable of nitrate reduction, although they often show negative results. In addition to these organisms, a group of closely related cultures received from H. L. Jensen of New South Wales, including some so-called 'Corynebacteria' from the soil, were tested using the medium recommended by Jensen (2) (see Table I). A much higher percentage of positive tests was found with this medium on all cultures than with the synthetic 'Manual' medium.

TABLE I  
SYNTHETIC NITRATE MEDIA

	Manual of methods	Jensen's medium	Proposed medium
K <sub>2</sub> HPO <sub>4</sub>	0.5 gm.	1.0 gm.	0.5 gm.
CaCl <sub>2</sub> (anhyd.)	0.5 gm.	—	—*
NaCl	—	0.5 gm.	0.5 gm.
MgSO <sub>4</sub> + 7 H <sub>2</sub> O	0.2 gm.	0.5 gm.	0.2 gm.
NaNO <sub>3</sub>	—	2.0 gm.	2.0 gm.
KNO <sub>3</sub>	1.0 gm.	—	—
Glucose	10.0 gm.	10.0 gm.	10.0 gm.
Agar	15.0 gm.	20.0 gm.	15.0 gm.
Distilled water	1000.0 cc.	1000.0 cc.	1000.0 cc.

\* If it is suspected that the organisms being studied require more calcium than is furnished by impurities present in the other ingredients, a small amount (not over 0.05 gm.) calcium chloride may be added.

With this in mind, a series of media were made up in order to determine, if possible, why Jensen's medium was superior. Using the 'Manual of Methods' medium as a basis, successive changes of one ingredient at a time were effected to make it, step by step, more like Jensen's medium. A change from potassium to sodium nitrate or from calcium to sodium chloride showed the greatest increase in the number of positive results. A new medium (see Table I, last column) was then tested with the media recommended in the Manual, using 65 organisms that included four spore-formers, three *Actinomyces* strains, 30 cultures showing the morphology typical of *Bacterium globiforme*, 23 other soil forms, three '*Corynebacterium*' cultures obtained from Jensen, and four

miscellaneous types. The new medium gave far superior results, that is, there were a greater number of nitrate positive organisms and a much smaller number of nitrate negative ones.

In order to determine why the new medium was better than that of the Manual of Methods, further tests were carried out. Because the greatest difference between the two media lay in the cations of the nitrate and the chloride, it appeared that the presence of sodium enhanced the production of nitrite in some way. Results, obtained when the amount of sodium or potassium was varied or when calcium was replaced by either one, indicated that the presence of both cations enhanced nitrate reduction even when calcium was used; but the best results were secured when sodium or potassium was substituted for calcium. It was noted that some precipitation of phosphate always occurred in the medium containing calcium, even when precautions were taken (as directed in the Manual) to dissolve the calcium salt and the phosphate separately; it was suspected, therefore, that phosphorus deficiency might be the cause of poor results obtained with it. To investigate this point, the same medium was made up without any phosphate, but with all other ions present; as was expected, growth was scanty and the percentage of positive reactions was very low.

It is apparent then that two factors are involved in the superiority of the proposed medium: first, both sodium and potassium ions are essential; second, too high a concentration of calcium precipitates the phosphates in the medium (as is visible in the preparation of the medium), thereby *interfering with nitrate reduction, even though enough phosphate remains available to permit what seems to the eye to be normal growth*. Complete elimination of the calcium ion is unnecessary as 0.05 gm. of calcium chloride per litre provides calcium but does not interfere with nitrate reduction. Inasmuch, however, as experiments show that the organisms included in this work do not need more calcium than they may be given in the form of impurities of the distilled water or 'chemically pure' salts, there seems to be no need for including it in the formula.

Accordingly, it is recommended that the synthetic nitrate medium of the Manual of Methods be replaced with the formula given in the last column of Table I, or at least with some similar formula calling for both sodium and potassium salts, but no calcium in any form. It must, of course, be recognized that this recommended medium, like the synthetic formula in the Manual of Methods is applicable only to those organisms that do not require organic nitrogen.

A search through the literature shows that many other media have been recommended for one purpose or another that calls for some phosphate together with comparatively large amounts of calcium. It is quite possible that an investigation of these media would show that bacteria growing in

them, also, suffer from phosphate deficiency sufficiently to interfere with their physiology, even though (like the organisms here investigated on this nitrate medium) their growth appears normal to the eye.

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